FORMULATION OPTIMIZATION AND CHARACTERIZATION OF IRINOTECAN NANOPARTICLES

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CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION, OPTIMIZATION AND CHARACTERISATION OF IRINOTECAN NANOPARTICLES"** submitted by **TREIVEDI SHAGUN MADHUR (26095159)** in partial fulfillment of the degree of Master of Pharmacy in Pharmaceutics of **THE TAMIL NADU DR.M.G.R MEDICAL UNIVERSITY**, Chennai at Annai Veilankanni's Pharmacy College, Chennai- 600 015 is the Bonafide work carried out by his/her under my guidance and supervision during the academic year 2010-2011. The dissertation or any part of this has not been submitted elsewhere for any other degree.

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The thesis entitled **"FORMULATION OPTIMIZATION AND CHARACTERIZATION OF IRINOTECAN NANOPARTICLES"** was carried out by the 26095159 in Department of Pharmaceutics, AnnaiVeilankanni's Pharmacy College, Saidapet, Chennai – 15 during the academic year 2010-2011. The work embodied in this thesis is original, and is not submitted in part or full for any other degree of this or any other University.

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TREIVEDI SHAGUN MADHUR

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LIST OF ABBREVIATIONS

Abbreviation	Expanded terminology
FDA	Food and drug administration
GIT	Gastro intestinal tract
UV	Ultra voilet
IRN	Irinotecan hydrochloride trihydrate
IRNNP	Irinotecan nanoparticles
USP	United state pharmacopoiea
PLGA	Poly lactide-o-gycolic acid
PVA	Poly vinyl alcohol
ACN	Acetonitrile
PDI	Poly dispersivity index
HPMA	Hydrolyzed Polymaleic Anhydride
ABI	Abraxane
DOX	Doxorubicin

LIST OF UNITS AND MEASUREMENTS

Units	Expanded terminology
%	Percentage
Mg	Milligram
Nm	Nanometer
µm	Micrometer
w/w	Weight by weight
w/v	Weight by volume
ml	millilitre
Min	Minutes
Hrs	Hours

ABSTRACT

The present research work is based on the formulation, optimization and characterization of nanoparticulate drug delivery system. The main rationale of the work is to formulate a targeted drug delivery system with enhanced drug entrapment efficiency. Here the drug of interest is Irinotecan hydrochloride trihydrate, an antineoplastic agent while the polymer used is poly lactide -o - glcolic acid (PLGA), a biodegradable polymer. The analytical method development is carried out using acetonitrile and phosphate buffer saline. The formulation optimization is also carried out optimizing its various process and formulation parameters. Different organic solvents were tried and various surfactants were used to optimize the nanoparticulate formulation. The size range and zeta potential was measured using Malvern zeta sizer. The lyofilization was carried out using two different cryoprotectants .

CHAPTER 1

INTRODUCTION

1.1 TARGETED DELIVERY OF ANTICANCER AGENTS

Much effort has been expended to improve the selectivity of cancer chemotherapeutic agents, and significant improvement in patient survival has been achieved in recent years. Nevertheless, the developments of novel selective agents and new ways of delivering both old and new agents are possibly the most important goals of modern anticancer research[82].

Targeted cancer chemotherapy aims to direct adequate concentration of the chosen agent to tumor cells while affecting as few healthy cells as possible. In principle, this can be achieved by passive or active targeting. Passive targeting exploits the enhanced permeability and retention (EPR) characteristics of tumor vessels. Rapidly growing tumors develop extensive vasculatures to meet their requirement for nutrient supply and waste disposal, but the blood vessels are abnormally hyper-permeable, with defective architecture and impaired lymphatic drainage. Circulating macromolecular drugs or particulate delivery systems that have difficulty permeating normal blood vessels can extravasate through such tumor blood vessels, and they become entrapped due to the impaired lymphatic drainage in tumor tissues[61].

Consequently, the EPR effect can be applied to facilitate the selective accumulation of an appropriately designed drug delivery system at a tumor site. To achieve efficient accumulation, the delivery system must also avoid systemic clearance by the reticuloendothelial system (RES), usually achieved by controlling the size and surface properties of the delivery systems. To avoid RES uptake, a hydrophilic surface and small particle size under 100 nm are the most often mentioned requirements). Active targeting, on the other hand, is often achieved by

exploiting the differences in membrane biochemistry between cancer and normal cells. Active targeting of a drug to cancer cells may involve the conjugation of tissue- or cell-selective ligands that bind specifically with receptors on the surface of tumor cells, examples of which include lectin-carbohydrate and antibody-antigen interactions. Selectivity is ensured by choosing ligands that bind to antigens or receptors that are either uniquely expressed or over-expressed on the target cells compared to normal tissues. To increase the payload to be delivered to the tumor site, the drug may be concentrated in a carrier, e.g. nanoparticles, which is then conjugated with the targeting ligand[61].

The concept of drug targeting, suggested by **Paul Ehrlich** almost a century ago, considered a hypothetical 'magic bullet' as an entity consisting of two components — the first one should recognize and bind the target, while the second one should provide a therapeutic action in this target. Currently, the concept of magic bullet includes a coordinated behavior of three components: (a) drug; (b) targeting moiety; and (c) pharmaceutical carrier used to multiply the number of drug molecules per single targeting moiety. Pharmaceutical carriers include soluble polymers, microcapsules, microparticles, cells, cell ghosts, lipoproteins, liposomes, and micelles. All of them can be made targeted in one-way or another[30,82] .

The recognition of the target can occur on the level of a whole organ, on the level of certain cells specific for a given organ, or even on the level of individual components characteristic of these cells, such as cell surface antigens. The most universal form of target recognition is the recognition on the molecular level, based on the fact that every organ or tissue certain compounds (antigens) can be found that are specific only for the organ of interest. For successful targeting, another compound can be used as a transporting unit, which is capable of the specific interaction with the specific target component (for example, a monoclonal antibody against the target antigen). Basing on this principle, numerous systems for drug targeting have been constructed capable of the delivery of pharmaceuticals to the variety of tissues and organs[30].

1.2 NANOPARTICULATE SYSTEMS FOR DRUG DELIVERY

Nanoparticles are engineered submicron-sized systems that range in size from a few nanometers to several hundred nanometers depending on their intended use. A variety of organic and inorganic materials, including polymers, lipids, ceramic and metals, have been used to construct nanoparticles. Most inorganic nanoparticles have a central core (usually metallic) and a protective organic surface coating. Organic nanoparticles include liposomes and other lipid-based carriers, polymeric nanoparticles, micelles and various ligand-targeted products. Structurally, nanoparticles have also been classified as dendrimers, micelles, nanospheres, nanocapsules, liposomes, fullerenes and nanotubes. Based on their manufacturing methods and materials used, the size and shape of nanoparticles vary.

Therapeutic drugs may be incorporated into nanoparticles by surface attachment or encapsulation. Nanoparticulate drug delivery systems are highly versatile. Drug payloads range from small molecular weight drugs to macromolecules, from highly water-soluble agents to strongly hydrophobic drugs. The method of delivery may vary from the simple, localized delivery using a catheter-based approach to sophisticated targeted delivery whereby the conjugation of biospecific ligand onto the nanoparticle surface could direct drug delivery to the tissue of interest. In addition, the small particle size of nanoparticles yields a high surface area per unit weight ratio that can greatly facilitate drug dissolution and absorption in the gastrointestinal fluids. Nanoparticulate systems have been demonstrated to improve drug bioavailability, facilitate drug solubilization, sustain drug effect in target tissues and improve the stability of therapeutic agents. Since the latter half of the 1980s, nanoparticles have been studied as carriers for drug delivery to challenge many diseases, including cancer, HIV, and diabetes. Much of the research has concentrated on improving the bioavailability of drugs with poor absorption characteristics and providing controlled release of drugs[46]. Therapeutic agents of interest are incorporated into polymer nanoparticles either by physical entrapment within the polymeric matrix or by surface adsorption or conjugation. The size and surface properties of the nanoparticles determine their fate in the human body. Unless there is intended drug delivery to the RES, the size and surface of the

nanoparticles must be designed to avoid RES clearance. Nanoparticles smaller than 100 nm in diameter have been found advantageous in this respect. So is the coating of nanoparticles with the hydrophilic polymer, polyethylene glycol (PEG) Targeted drug delivery is realized by surface conjugation with a biospecific ligand, which may also favorably modify the intracellular disposition of the nanoparticles. Biocompatible, hydrophilic or hydrophobic polymer nanoparticles with surface-pendant amine, carboxyl or aldehyde groups have been fabricated for further bio-conjugation. A wide variety of ligands, such as folic acid, antibody, and aptamers have been used for surface modification of polymeric nanoparticles to impart cancer cell targeting capability.

Polymers employed for nanoparticle fabrication have included synthetic polymers, such as Poly (D, L-lactic-co-glycolic acid) (PLGA), polyacrylates and polycaprolactones, and natural polymers. In particular, the application of polymer nanoparticles in oncology has grown greatly with the advent of biodegradable polymers. Biodegradable polymers are macromolecular materials capable of being degraded into simpler products through chemical or enzyme-catalyzed hydrolysis in the body. Biodegradable and biocompatible polymers as drug carriers are desirable to minimize toxicity and avoid the requirement to surgically remove the spent carriers. For these reasons, nanoparticles made of biodegradable materials are often fabricated to provide sustained drug release within the target site. A good example of a biodegradable and biocompatible polymer is PLGA**,** an FDA-approved biodegradable and biocompatible polymer for biomedical applications[46].

CHAPTER 2

LITERATURE REVIEW

2.1 CANCER

Alaa Eldeen B.et al., defined that cancer is the uncontrolled growth of abnormal cells in the body. Cancerous cells are also called malignant cells. Cancer grows out of normal cells in the body. Normal cells multiply when the body needs them, and die when the body doesn't need them. Cancer appears to occur when the growth of cells in the body is out of control and cells divide too quickly. It can also occur when cells forget how to die. A protein called DNA very carefully controls this growth, which is contained in genes. It is this information that allows for healing of a wound[4].

Surendrian A. et al., suggested that if cells in a body divide only when necessary and stop when the job is done the total number of cells in the adult body will remain constant. However when a cell starts dividing and when the daughter cells in turn keep dividing without any purpose, then the number of cells in that particular area begins to increase and a lump appears - a cancer[1]**.**

A complex event usually involves many causal factors, which in turn are causally linked to other factors. In talking about cause and effect, we customarily designate as its cause one or a few salient factors most directly connected to its salient effects, noting a few other factors as indirect causes, relegating many factors as background conditions, and ignoring factors too vague to determine. For example, when arson investigators decide that a fire was caused by sparks from an exposed electric wire, they treat as background conditions the presence of oxygen and flammable materials nearby. What made the wire exposed they regard as indirect causes, which may interest criminal investigators. Saliency and relevancy are context dependent

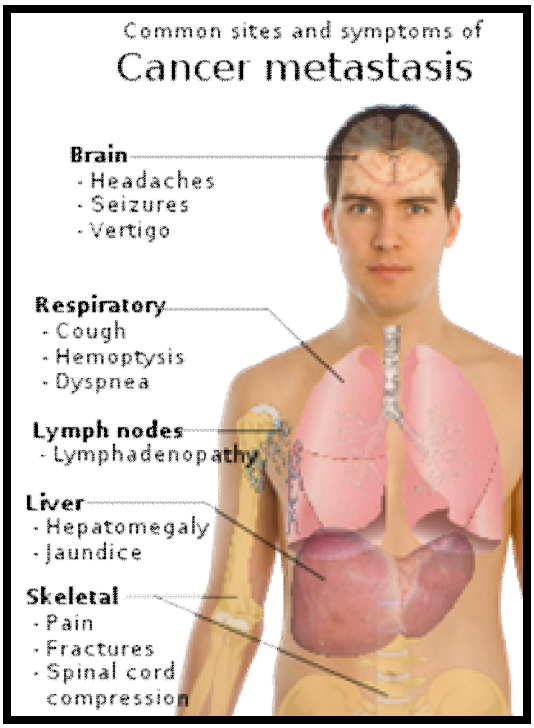

Fig 2.1 Common sites and symptoms of cancer metastasis

Douglas and David showed that in cancer research, two scientific approaches operate in two general kinds of context. Epidemiological focuses on causal factors on the levels of people and population, with results that are more useful for disease prevention. Molecular cell biology focuses on causal mechanisms on the levels of genes and cells, with results that are more useful to treatment and cure. To biologists, factors identified by epidemiology are indirect causes in the mechanism of cancer development. For most people, however, these factors are the only cancer causes that they care about; they can do something about them[18,20].

No treatment, conventional or otherwise, can completely eliminate all cancer cells according to the naturally oriented physician. The reason is simple. Cancer is a systemic disease, and there are simply too many cancerous or pro-

cancerous cells within the ecosystem of the body. Cancer is not a localized problem but a whole-body phenomenon of metastatic growth. Its growth process is affected by biological conditions. Non-genetically based cancer forms in the body because of toxins, the lack of oxygen poor nutrition, and other factors such as hormonal imbalance. Whether the cancer in our body continues to multiply depends to a large degree on our body's biological terrain. It is this terrain that determines how the cancer is expressed[20].

2.1.1 How cancer spreads:

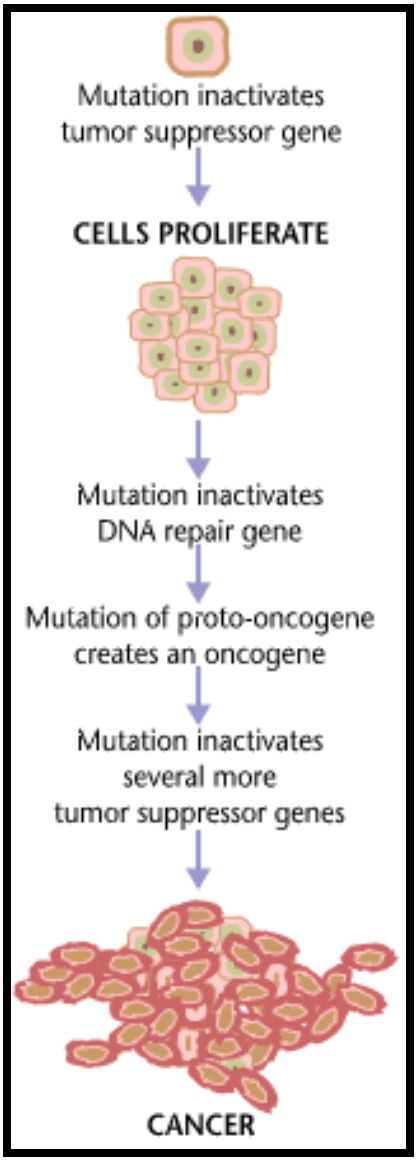

Fig 2.2 Stages of tumor development

At the center of cellular proliferation is the cell division cycle, the process by which a cell grows, replicates its DNA and then divides to give two daughter cells. This process is divided into four sequential phases (Figure 1.). It is often considered that the two most important of these are S phase, when DNA replication occurs and mitosis (also known as M phase), when the cell undergoes division to give two daughter cells. In fact a key concept of the cell cycle is that S phase must always follow M phase and that M phase must not start until S phase has been completed[50]. In other words, DNA replication must not commence until mitosis is complete and mitosis must not begin until the previous round of DNA replication has ended, thus, the integrity of the genome is maintained. In-between S and M phase are two gaps G1 and G2. G1 follows on from mitosis and is a time during the cell cycle when the cell is responsive to both positive and negative growth signals. G2 is the gap after S phase, when the cell prepares for entry into mitosis. Finally, there is a fifth state, G0 (also known as quiescence) into which the cell may reversibly exit from G1, if it is deprived of the appropriate growth-promoting signals[50,51].

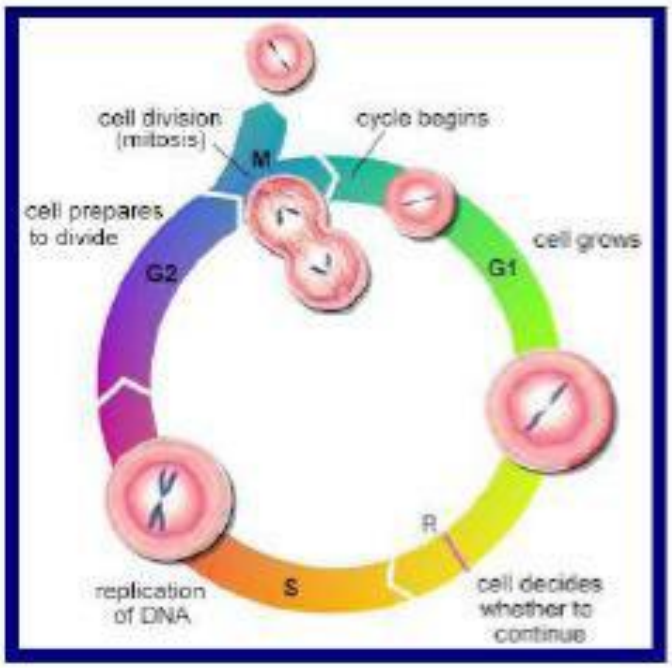

Figure2.3: Cancer cell cycle

All cancers begin in cells, the body's basic unit of life. To understand cancer, it's helpful to know what happens when normal cells become cancer cells.

The body is made up of many types of cells. These cells grow and divide in a controlled way to produce more cells, as they are needed to keep the body healthy. When cells become old or damaged, they die and are replaced with new cells.

However according to ***national cancer institute*** U.S., sometimes this orderly process goes wrong. The genetic material (DNA) of a cell can become damaged or changed, producing mutations that affect normal cell growth and division. When this happens, cells do not die when they should and new cells form when the body does not need them. The extra cells may form a mass of tissue called a tumor[80].

If the cells that formed the cancerous lump remained only at that site then most cancers could be treated effectively. But cancers cells have another dangerous feature - they can spread. As the cancer grows clumps of cells will invade tissues around them and later invade arteries and veins and can be carried along by them. They could settle in another site far away from the original site. Cancers can also travel along channels called lymphatic and spread to lymph nodes[10,21].

2.1.2 Primary and secondary cancer

The site where the cancer started is called the primary cancer. The site the cancer spreads to be called the secondary cancer.

For example a cancer may start in the breast. This is called a primary breast cancer. It may spread to the liver and lungs. These are not new cancers in the liver and lungs but secondaries from the primary breast cancer[27].

2.1.3 Oncogenes

No one knows what it is that transforms a normal cell into a cancerous cell. We know however that there are certain genes-oncogenes that can either promote or

suppress cell division. P53 is a suppressor oncogene. In certain breast cancer tissues excessive amounts of a defective P53 gene are present.

These cancers are generally believed to be aggressive. It may be possible in the future to regulate growth of cancer cells by modifying these genes[27].

2.1.4 The causes

Likely cause of cancer	Estimated proportion of cancer deaths (%)
Environmental	
Cigarette smoking	35
Diet	2
Occupation	2
Background radiation	5
Non-environmental	
Generic	5
Total	74
Unaccounted	26

Table 2.1 Causes of cancer

From the table 1.1 above we see that almost 70% of cancers are believed to be caused by environmental factors - which mean these cancers should be preventable. However it is not always possible to link a particular cancer causing substance (carcinogen) and a cancer, as there is usually a long interval – latent period - between the exposure and the cancer.

Surendrian A. et al., explained many different kinds of cancers. Cancer can develop in almost any organ or tissue, such as the lung, colon, breast, skin, bones, or nerve tissue[1].

There are also other causes of cancers, including:

- Benzene and other chemicals
- Drinking excess alcohol
- Environmental toxins, such as certain poisonous mushrooms and a type of poison that can grow on peanut plants (aflatoxins)
- Excessive sunlight exposure
- Genetic problems
- Obesity
- Radiation
- Viruses

However, the cause of many cancers remains unknown.

2.1.5 Types of Cancer Classified by Body System

Cancer has the potential to affect every organ in the body. The cells within malignant tumors have the ability to invade neighboring tissues and organs, thus spreading the disease. It is also possible for cancerous cells to break free from the tumor and enter the bloodstream, in turn spreading the disease to other organs. This process of spreading is called metastasis. When cancer has metastasized and has affected other areas of the body, the disease is still referred to the organ of origination. For instance, if cervical cancer spreads to the lungs, it is still called cervical cancer, not lung cancer[65].

The most common types of cancer in the United States based on frequency of diagnosis are:

- Bladder cancer
- Breast cancer
- Colon cancer

- Endometrial cancer
- Kidney cancer (renal cell)
- Leukemia
- Lung cancer
- Melanoma
- Non-Hodgkin lymphoma
- Pancreatic cancer
- Prostate cancer

2.1.5.1 Blood Cancer

The cells in the bone marrow that give rise to red blood cells, white blood cells, and platelets can sometimes become cancerous. These cancers are leukemia or lymphoma[65].

- Leukemia
- Lymphoma
- Multiple Myeloma
- Waldenstrom's Macroglobulinemia

2.1.5.2 Bone Cancer

Bone cancer is a relatively rare type of cancer that can affect both children and adults, but primarily affects children and teens. There are several types of bone cancer, but the most common types are:

- Ewing's Sarcoma
- Osteosarcoma

2.1.5.3 Brain Cancer

Brain tumors can be malignant (cancerous) or benign (non-cancerous). They affect both children and adults. Malignant brain tumors don't often spread beyond the brain. However, other types of cancer have the ability to spread to the brain[69]. Types of brain cancer include:

- Adult Brain Tumor
- Brain Stem Glioma, Childhood
- Cerebellar Astrocytoma, Childhood
- Cerebral Astrocytoma/Malignant Glioma, Childhood
- Ependymoma, Childhood
- Medulloblastoma, Childhood
- Supratentorial Primitive Neuroectodermal Tumors and Pineoblastoma, Childhood
- Visual Pathway and Hypothalamic Glioma, Childhood.

2.1.5.4 Breast Cancer

Breast cancer is a common type of cancer that affects women and much less commonly, men. More than 200,000 women are diagnosed with breast cancer in the United States each year[65]. Types of breast cancer include, but are not limited to:

- Ductal carcinoma in situ
- Lobular carcinoma in situ
- Inflammatory breast cancer
- Paget's disease of the nipple
- Invasive types of breast cancer

2.1.5.5 Digestive/Gastrointestinal Cancers

This is a broad category of cancer that affects everything from the esophagus to the anus. Each type is specific and has its own symptoms, causes, and treatments[69].

- Anal Cancer
- Bile Duct Cancer, Extrahepatic
- Carcinoid Tumor, Gastrointestinal
- Colon Cancer
- Esophageal Cancer
- Gallbladder Cancer
- Liver Cancer, Adult Primary
- Liver Cancer, Childhood
- Pancreatic Cancer
- Rectal Cancer
- Small Intestine Cancer
- Stomach (Gastric) Cancer

2.1.5.6 Endocrine Cancers

The endocrine system is an instrumental part of the body that is responsible for glandular and hormonal activity. Thyroid cancer is the most common of the endocrine cancer types and generally, the least fatal[65].

- Adrenocortical Carcinoma
- Carcinoid Tumor, Gastrointestinal
- Islet Cell Carcinoma (Endocrine Pancreas)
- Parathyroid Cancer
- Pheochromocytoma

- Pituitary Tumor
- Thyroid Cancer

2.1.5.7 Eye Cancer

Like other organs in the human body, the eyes are vulnerable to cancer as well. Eye cancer can affect both children and adults.

- Melanoma, Intraocular
- Retinoblastoma

2.1.5.8 Genitourinary Cancers

These types of cancer affect the male genitalia and urinary tract[65].

- Bladder Cancer
- Kidney (Renal Cell) Cancer
- Penile Cancer
- Prostate Cancer
- Renal Pelvis and Ureter Cancer, Transitional Cell
- Testicular Cancer
- Urethral Cancer
- Wilms' Tumor and Other Childhood Kidney Tumors

2.1.5.9 Gynecologic Cancers

This group of cancer types affects the organs of the female reproductive system. Specialized oncologists called gynecologic oncologists are recommended for treating gynecologic cancer.

- Cervical Cancer
- Endometrial Cancer
- Gestational Trophoblastic Tumor

- Ovarian Cancer
- Uterine Sarcoma
- Vaginal Cancer

2.1.5.10 Head and Neck Cancer

Most head and neck cancers affect moist mucosal surfaces of the head and neck, like the mouth, throat, and nose. Causes of head and neck cancer vary, but cigarette smoking plays a role. Current research suggests a strong HPV link in the development of some head and neck cancer[69].

- Hypopharyngeal Cancer
- Laryngeal Cancer
- Lip and Oral Cancer
- Metastatic Squamous Neck Cancer
- Nasopharyngeal Cancer
- Oropharyngeal Cancer
- Paranasal Sinus and Nasal Cavity Cancer
- Parathyroid Cancer
- Salivary Gland Cancer

2.1.5.11 Respiratory Cancers

Cigarette smoking is the primary cause for cancer affecting the respiratory system. Exposure to asbestos is also a factor[65].

- Lung Cancer, Non-Small Cell
- Lung Cancer, Small Cell
- Malignant Mesothelioma
- Thymoma and Thymic Carcinoma

2.1.6 Diagnosis

To confirm a diagnosis of cancer it is almost always necessary to remove a small piece of the tumor for examination; this is called a biopsy. The presence in the blood of certain proteins called tumor markers (e.g. C.E.A., CA-125, P.S.A. etc.) may point to a diagnosis of cancer but are never enough in themselves to establish the diagnosis. Once the diagnosis is made, it may be necessary to carry out further tests such as CT Scans, Bone marrow studies etc. to see if there is evidence of spread of cancer. This is called staging[13].

2.1.7 Treatment

Treatment of cancer is by surgery, radiotherapy and chemotherapy.

2.1.7.1 Surgery

In cancer surgery is used

1) To make the diagnosis
2) To remove all the tumor or most of the tumor
3) To control symptoms even when a cancer is too advanced to be cured e.g. removing a tumor that is causing severe bowel obstruction and lastly
4) To prevent cancers by removing an organ when the risk of malignant transformation is very high.

2.1.7.2 Radiotherapy

This is the use of x-rays to treat cancers. Some cancers are curable by radiotherapy e.g. cancers of the cervix. It is also used to mop up the tumor area after surgery. In incurable cancers radiotherapy can provide relief from pain caused by spread to the bones[13].

2.1.7.3 Chemotherapy

This involves the use of drugs to destroy cancer cells. Some cancers are curable by chemotherapy e.g. certain lymphomas. In certain cancers especially breast and colon cancers chemotherapy is used in addition to surgery and radiotherapy to prevent spread of the cancers[13].

2.2 ROLE OF NANOTECHNOLOGY IN CANCER THERAPY

Ferrai M. explained that the nanomedicine involves utilization of nanotechnology for the benefit of human health and well being. The use of nanotechnology in various sectors of therapeutics has revolutionized the field of medicine where nanoparticles of dimensions ranging between 1 - 100 nm are designed and used for diagnostics, therapeutics and as biomedical tools for research. It is now possible to provide therapy at a molecular level with the help of these tools, thus treating the disease and assisting in study of the pathogenesis of disease[22]. Conventional drugs suffer from major limitations of adverse effects occurring as a result of non-specificity of drug action and lack of efficacy due to improper or ineffective dosage formulation (*e.g*., cancer chemotherapy and antidiabetic agents). Designing of drugs with greater degree of cell specificity improves efficacy and minimizes adverse effects. Diagnostic methods with greater degree of sensitivity aid in early detection of the disease and provide better prognosis. Nanotechnology is being applied extensively to provide targeted drug therapy, diagnostics, tissue regeneration, cell culture, biosensors and other tools in the field of molecular biology. Various nanotechnology platforms like fullerenes, nanotubes, quantum dots, nanopores, dendrimers, liposomes, magnetic nanoprobes and radio controlled nanoparticles are being developed[10].

2.2.1 Current status of therapeutics

The major factors influencing the treatment outcome in a patient are the efficacy and safety profile of the drug more so when used for cancer chemotherapy. These drugs have poor cell specificity and high toxicity like bone marrow suppression, gastric erosion, hair loss, renal toxicity, cardiomyopathy, and several

effects on other systems. Similarly treatment for diabetes faces challenges with the route of delivery and inadequate glycaemic control. Availability of non-parenteral dosage forms of insulin would be a breakthrough and development of a suitable drug delivery device can aid in this approach. In many cases, the sensitivity and specificity of various diagnostic methods as in radio imaging and various assays for detection of malignancy are not sufficient enough for early detection and treatment[1].

2.2.2 Liposomes

Liposomes discovered in mid 1960s were the original models of nanoscaled drug delivery devices. They are spherical nanoparticles made of lipid bilayer membranes with an aqueous interior but can be unilamellar with a single lamella of membrane or multilamellar with multiple membranes. They can be used as effective drug delivery systems. Cancer chemotherapeutic drugs and other toxic drugs like amphotericin and hamycin, when used as liposomal drugs produce much better efficacy and safety as compared to conventional preparations. These liposomes can be loaded with drugs either in the aqueous compartment or in the lipid membrane. Usually water-soluble drugs are loaded in aqueous compartment and lipid soluble drugs are incorporated in the liposomal membrane[73,74]. The major limitation of liposome is its rapid degradation and clearance by the liver macrophages, thus reducing the duration of action of the drug it carries. This can be reduced to a certain extent with the advent of stealth liposomes where the liposomes are coated with materials like polyoxyethylene that prevents opsonisation of the liposome and their uptake by macrophages. Other ways of prolonging the circulation time of liposomes are incorporation of substances like cholesterol, polyvinylpyrollidone polyacrylamide lipids and high transition temperature phospholipids distearoyl phosphatidylcholine[74].

Targeting of liposomal drugs:

Liposomes can be targeted to specific organ or tissue by passive as well as active methods. As the liposomal drug acts minimally on other tissues, the safety profile is better than non-liposomal drug. The vascularity in tumour tissue is poorly

organized and significant leak occurs from blood vessel in the tumour tissue. The liposomal drugs get accumulated in the tumour tissue passively and produce enhanced effects. Active targeting of the drug can be achieved by using immunoliposomes and ligand directed liposomes.

Sapra P. et al suggested that immunoliposomes are liposomes conjugated with an antibody directed towards the tumour antigen. The antibody can be conjugated to the surface of a stealth liposome, the polyoxyethylene coating of a stealth liposome or on the surface of a non-stealth liposome. These immunoliposomes when injected into the body, reaches the target tissue and gets accumulated in its site of action. This reduces unwanted effects and also increases the drug delivery to the target tissue, thus enhancing its safety and efficacy[73].

Antibody directed enzyme prodrug therapy (ADEPT) consists of liposomes conjugated with an enzyme to activate a prodrug and an antibody directed to a tumour antigen (enzyme linked immunoliposomes). These are administered prior to administration of a prodrug. The antibody directs the enzyme to the target tissue where it activates the prodrug selectively and converts it to its active form. This way, action of the drug is avoided in other normal tissues, thus minimizing the toxicity of drug.

Such studies are being tried with epirubicin and doxorubicin. Ligand bearing liposomes are conjugated with specific ligands, which are directed towards target structures. In ovarian cancer, over expression of folate receptors by the tumour tissue occurs. The liposomal drug can be conjugated with folate so as to direct the molecule to the tumour. This method is also being tried in the treatment of leishmaniasis where liposomal hamycin conjugated with mannosyl human serum albumin are targeted towards human macrophages. Asialofeutin conjugation is being tried to target liver cells for gene therapy. The targeted liposomal preparations are found to have a better efficacy than non-targeted liposomes[73,74].

2.2.3 Polymeric Nanoparticles

To reach the targeted tumor tissue, nanoparticles must be able to stay in the bloodstream for considerable lengths of time without being eliminated. Nanoparticles with no surface modification are usually caught by the MPS, primarily the liver and spleen, during circulation, depending on their size and surface characteristics. To overcome this problem, nanoparticles can be coated with hydrophilic polymers. Coating can efficiently protect nanoparticles from capture by macrophages. The increased hydration also helps nanoparticles to be more water soluble and less sensitive to enzymatic degradation, therefore enhancing biocompatibility.

During the past decade, the application of polymer-based drug delivery systems in oncology has grown exponentially with the advent of biodegradable polymers. In these polymers, drugs are either physically dissolved, entrapped, encapsulated, or covalently attached to the polymer matrix. The resulting compounds may have different structures, including micelles and dendrimers. Both natural (albumin, chitosan, heparin, etc.) and synthetic (poly-L-lactide, poly-[Lglutamate], poly-[D,L-lactide-co-glycolide], PEG, etc.) biodegradable polymers are being exploited as drug delivery system[92].

Recently, a nanoparticle formulation of paclitaxel bound to albumin (Abraxane or ABI-007) was approved for the treatment of metastatic breast cancer. In a Phase III clinical trial, ABI-007 showed greater therapeutic efficacy and increased response compared with free paclitaxel. Currently, more than 10 formulations of anticancer polymeric nanoparticles have entered clinical development, including paclitaxel poliglumex (Xyotax), N- (2-hydroxypropyl) methacrylamide (HPMA) copolymer-camptothecin (MAG-CPT), and HPMA-DOX (PK1). In Phase I/II clinical trials, HPMA-DOX showed a 4 to 5-fold reduction in anthracycline-related toxicity. At DOX equivalent doses of 80 to 320 mg/m2, the drug still displayed significant antitumor activity in chemotherapy-refractory patients (including those with breast cancer). A recent Phase III trial showed that paclitaxel poliglumex (Xyotax) was less toxic than free paclitaxel and could prolong the survival of non-small-cell lung cancer patients with poor performance status. Also, paclitaxel poliglumex can be used as a novel radiation sensitizer[89].

2.2.4 Targeting mechanism

In principal, nanoparticle delivery of anticancer drugs to tumor tissues can be achieved by either passive or active targeting.

2.2.4.1 Passive Targeting

Passive targeting takes advantage of the inherent size of nanoparticles and the unique properties of tumor vasculature, such as the enhanced permeability and retention (EPR) effect and the tumor microenvironment. This approach can effectively enhance drug bioavailability and efficacy.

EPR Effect*:* Angiogenesis is crucial to tumor progression. Angiogenic blood vessels in tumor tissues, unlike those in normal tissues, have gaps as large as 600 to 800 nm between adjacent endothelial cells. This defective vascular architecture coupled with poor lymphatic drainage induces the EPR effect, which allows nanoparticles to extravasate through these gaps into extravascular spaces and accumulate inside tumor tissues. Dramatic increases in tumor drug accumulation, usually of 10-fold or greater, can be achieved when a drug is delivered by a nanoparticle rather than as a free drug.

However, the localization of nanoparticles within the tumor is not homogeneous. The factors that result in high concentrations of nanoparticles in one part of the tumor tissue but not in other parts are not well understood yet. In general, the accumulation of nanoparticles in tumors depends on factors including the size, surface characteristics, and circulation half-life of the nanoparticle and the degree of angiogenesis of the tumor. Usually, less nanoparticle accumulation is seen in preangiogenic or necrotic tumors[71].

Although PLGA represents the ‘gold standard’ (exemplified by more than 500 patents) of biodegradable polymers, increased local acidity because of degradation can lead to irritation at the site of polymer application. Further, the increased local acidity may also be detrimental to the stability of protein drugs[62].

Tumor Microenvironment

Hyperproliferative cancer cells have profound effects on their surrounding microenvironment. Tumors must adapt to use glycolysis (hypoxic metabolism) to obtain extra energy, resulting in an acidic microenvironment. In addition, cancer cells overexpress and release some enzymes that are crucial to tumor migration, invasion, and metastasis, including matrix metalloproteinases (MMPs). Tumoractivated prodrug therapy is an example of passive targeting that takes advantage of this characteristic of the tumor-associated microenvironment. A nanoparticle conjugating an albumin-bound form of DOX with an MMP-2–specific peptide sequence (Gly-Pro-Leu-Gly-Ile-Ala-Gly-Gln) was efficiently and specifically cleaved by MMP- 2. When certain pH-sensitive molecules are incorporated into liposomes, drugs can be specifically released from the complexes by a change in pH.9. The pH-sensitive liposomes are stable at physiologic conditions (pH 7.2), but degraded in tumor-associated acidic areas.

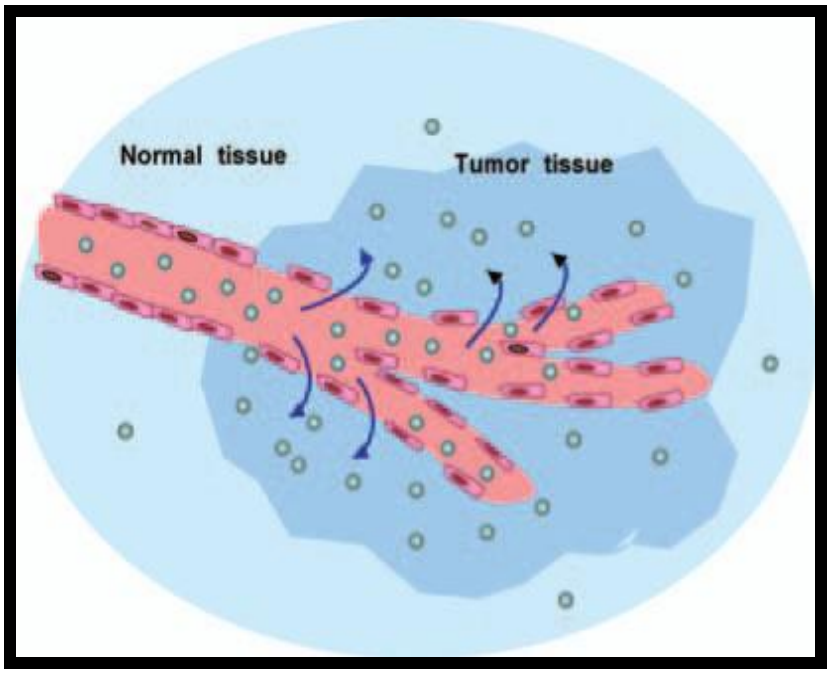

Fig 2.4: Passive Tumor Targeting with Nanoparticle Drugs. Long-circulating therapeutic nanoparticles accumulate passively in solid tumor tissue by the enhanced permeability and retention effect. The hyperpermeable angiogenic tumor vasculature allows preferential extravasation of circulating nanoparticles.

Likewise, thermolabile liposomes were expected to be activated by the local hyperthermic microenvironment[71].

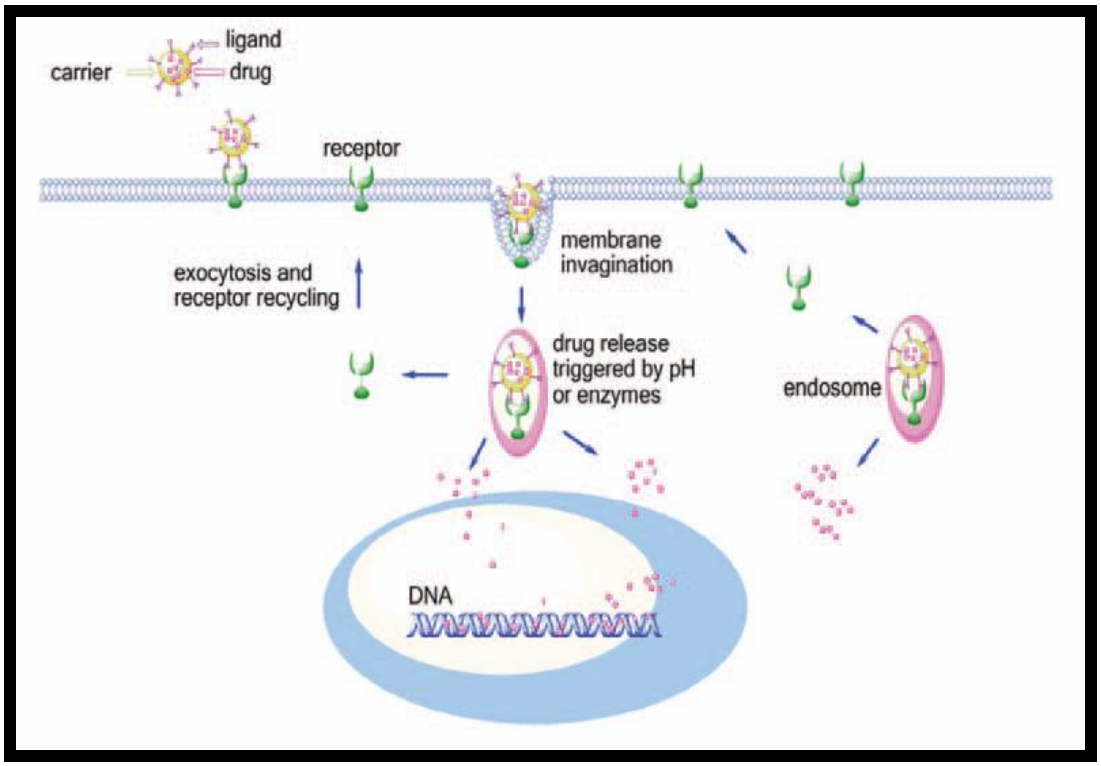

Fig 2.5 Internalization of Nanoparticles via Receptor-mediated Endocytosis. Nanoparticle-conjugated tumor-specific ligands/antibodies bind to surface receptors, triggering nanoparticle internalization through an endosome-dependent mechanism. As the interior of the endosome becomes more acidic, drugs are released from the nanoparticle into the cytoplasm.

2.2.4.2 Active Targeting

The polymeric nanoparticles that have been tested clinically so far have mostly lacked a targeting moiety and instead rely mainly on the EPR effect of tumors, the tumor microenvironment, and tumor angiogenesis to promote some tumorselective delivery of nanoparticles to tumor tissues.

However, these drug delivery systems using a binary structure conjugate inevitably have intrinsic limitations to the degree of targeting specificity they can achieve. In the case of the EPR effect, while poor lymphatic drainage on the one

hand helps the extravasated drugs to be enriched in the tumor interstitium, on the other hand, it induces drug outflow from the cells as a result of higher osmotic pressure in the interstitium, which eventually leads to drug redistribution in some portions of the cancer tissue[73].

Garrett explained an alternative strategy to overcome these limitations is to conjugate a targeting ligand or an antibody to nanoparticles. By incorporating a targeting molecule that specifically binds an antigen or receptor that is either uniquely expressed or overexpressed on the tumor cell surface, the ligand-targeted approach is expected to selectively deliver drugs to tumor tissues with greater efficiency (Figure 2). Such targeted nanoparticles may constitute the next generation of polymeric nanoparticle drug delivery systems. Indeed, several targeted polymeric nanoparticles are currently undergoing preclinical studies[16].

2.2.4.2.1 Choice of Target Receptor

Selection of the appropriate receptor or antigen on cancer cells is crucial for the optimal design of targeted nanoparticles. The ideal targets are those that are abundantly and uniquely expressed on tumor cells, but have negligible or low expression on normal cells. The targeted antigen or receptor should also have a high density on the surface of the target tumor cells. Whether the targeted nanoconjugate can be internalized after binding to the target cell is another important criterion in the selection of proper targeting ligands. In the case of an antibody or other ligand that cannot trigger the internalization process, the drug can enter cells through simple diffusion or other transport system after being released from the targeted conjugate at or near the cell surface[63]. However, drug released outside the cell may disperse or redistribute to the surrounding normal tissues rather than exclusively to the cancer cells. *In vitro* and *in vivo* comparisons using internalizing or noninternalizing ligands have shown that the intracellular concentration of drug is much higher when the drug is released from nanoparticles in the cytoplasm after internalization[74].

2.2.4.2.2 Choice of Targeting Ligand

One of the greatest challenges to the design of nanoparticles that can selectively and successfully transport drug to cancerous tissues is the choice of targeting agent(s). This strategy also relies on the ability of the targeting agent or ligand to bind the tumor cell surface in an appropriate manner to trigger receptor-mediated endocytosis. The therapeutic agent will thereby be delivered to the interior of the cancer cell. A variety of tumor targeting ligands, such as antibodies, growth factors, or cytokines, have been used to facilitate the uptake of carriers into target[51].

Ligands targeting cell-surface receptors can be natural materials like folate and growth factors, which have the advantages of lower molecular weight and lower immunogenicity than antibodies. However, some ligands, such as folate that is supplied by food, show naturally high concentrations in the human body and may compete with the nanoparticle-conjugated ligand for binding to the receptor, effectively reducing the intracellular concentration of delivered drug.

Recent advances in molecular biology and genetic engineering allow modified antibodies to be used as targeting moieties in an active-targeting approach. MAbs or antibody fragments (such as antigen-binding fragments or single-chain variable fragments) are the most frequently used ligands for targeted therapies. Whole mAbs have 2 binding domains showing high binding avidity. The Fc domain of the mAb can induce complement-mediated cytotoxicity and antibody- dependent, cell-mediated cytotoxicity, leading to additional cell-killing effect. On the other hand, the Fc domain also initiates an immune response and can be rapidly eliminated in the circulation, resulting in decreased accumulation of targeted nanoparticles into cancer cells[51].

Compared with whole mAbs, the use of antibody fragments as a targeting moiety can reduce immunogenicity and improve the pharmacokinetic profiles of nanoparticles. For example, liposomes coupled with mAb fragments instead of whole antibodies showed decreased clearance rates and increased circulation half-lives, allowing the liposomes sufficient time to be distributed and bind to the

targeted cells. This strategy improved the therapeutic efficacy of immunoliposomal DOX targeted against CD19 on human B lymphoma cells in animal models.

2.2.4.2.3 Tumor extra cellular pH targeting:

Tumor-targeting approaches have been developed for improved efficacy and reduced toxicity by altering biodistribution of cancer drugs and by using specific cell surface interactions. Solid tumors are often characterized by over expression of specific antigens or receptors on cell surfaces and Antigens and receptors help in transmitting signals from the surrounding environment that are essential for the growth of tumor cells. Targeting antigens or receptors has been extensively investigated as an important delivery mode by using macromolecular or nano-sized carriers to tumor cells. Nanodrug carriers attached with surface ligands or antibodies exploit these receptor-mediated uptake pathways that are recognized and internalized by the tumor cells[85].

However, these approaches have achieved limited success in clinic, most likely because of significant heterogeneity in both solid tumor cell types and cell surface markers [8] N. Scholler, N. Fu, Y. Yang, Z. Ye, G.E. Goodman, K.E. Hellstrom and I. Hellstom, Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma, *Proc. Natl. Acad. Sci. U. S. A.* **96** (1999), pp. 11531–11536. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (140). Additionally, both the presence of antigens and the expression of receptors on surface of these tumor cells are transient and dynamic. The heterogeneity of cancer cells may explain the reasons for the unexpected results of targeting strategy[6.81].

The extracellular pH (pH_e) of normal tissues and blood pH are kept constant at pH 7.4 and their intracellular pH (pH_i) at 7.2. However, in most tumors the pH gradient is reversed ($pH_i > pH_e$). Particularly, tumor pH_e is lower than normal tissues. Although there is a distribution in *in vivo*, pH_e measurements made by using needle type microelectrodes on human patients having various solid tumors

(adenocarcinoma, squamous cell carcinoma, soft tissue sarcoma, and malignant melanoma) and in readily accessible areas (limbs, neck, or chest wall), shows the mean pH value to be 7.0 with a range between 5.7 and 7.8.

This variation is dependent upon tumor histology, tumor volume, and location inside a tumor[81]. Recent measurements of pH_e by noninvasive technology such as ^{19}F, ^{31}P, or ^{1}H probes by magnetic resonance spectroscopy in human tumor xenografts and in animals further proved consistently low pH_e. Reported pH_e data on human and animal solid tumors either by invasive or noninvasive methods showed that more than 80% of all measured values are below pH 7.2. The primary reason for this imbalance in cancer pH is the high rate of glycolysis in cancer cells, both in aerobic and anaerobic conditions. It is also proposed that the acidic milieu benefits the cancer cells by generating an invasive environment that tears down the extracellular matrix and destroys the surrounding normal tissue cells[54].

There are a variety of mechanisms associated with MDR cells that need to be circumvented for a successful tumor treatment. At unicellular level, ATP dependent drug-efflux pumps of P-glycoprotein (Pgp), multidrug resistance protein (MRP), lung resistance protein (LRP), antiapototic (or survival) bcl-2 gene, and altered expression of Topoisomerase II interfere with a sufficient intracellular drug dose and decrease the effectiveness of drug in killing tumor cell. In clinical setting, additional tumor microenvironmental factors such as epidermal growth factor, fibroblast growth factor, insulin-like growth factor, and extracellular matrix components are strongly associated with survival mechanisms of cancer cells under cytotoxic drug treatment[51].

To tackle themultifacetedMDRmechanisms, it is hypothesized that a focal high dose strategy works at cellular level rather than at systemic level. In addition, local high dose may overwhelm most resistant mechanisms, which might have their intrinsic limitation in defense capability because even extremely resistant experimentalMDR cells are killed at high drug concentrations. Intracellular organelles in parental drug-sensitive cells are characterized to have somewhat acidic,

diffuse pH profiles inside cells[54]. MDR cancer cells develop more acidic organelles (recycling endosome and lysosome) than those in sensitive cells, which are more acidic than cytosolic pH and nucleoplasmic pH. This results in acid-induced sequestration of anticancer drugs. Acidic organelles in MDR cells contribute to developing resistance to chemotherapeutic drugs.

Since most anticancer drugs are in an ionizable form, the pH of extracellular matrix and intracellular compartments are critical factors in determining drug partitioning and distribution. The low pH in tumor extracellular space or in various subcellular organelles is a significant signal for targeting[89].

CHAPTER 3

AIM AND OBJECTIVE

Nanomedicine involves utilization of nanotechnology for the benefit of human health and well being. The use of nanotechnology in various sectors of therapeutics has revolutionized the field of medicine where nanoparticles of dimensions ranging between 1 - 100 nm are designed and used for diagnostics, therapeutics and as biomedical tools for research[1]. It is now possible to provide therapy at a molecular level with the help of these tools, thus treating the disease and assisting in study of the pathogenesis of disease[44].

The present work was carried out to develop a PLGA based nanoparticulate drug delivery system for an anticancer drug irinotecan hydrochloride trihydrate (IRN) for effecient tumor targeting.

The main objectives of this work are as follows:

a) Analytical method development

b) Formulation and optimization of IRN loaded PLGA nanoparticles.

c) Charaterization of IRN loaded PLGA nanoparticles.

d) In vitro drug release study.

CHAPTER 4

DRUG AND POLYMER PROFILE

4.1 DRUG PROFILE

4.1.1 Chemical name

Irinotecan hydrochloride trihydrate

4.1.2 Molecular structure

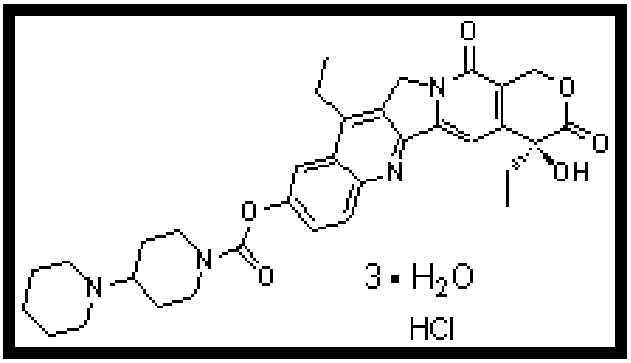

Figure 4.1: Molecular structure of IRN

4.1.3 Molecular formula

$C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3\ (H_2O)$

4.1.4 IUPAC Name

(19S)-10,19-diethyl-19-hydroxy-14,18-dioxo-17-oxa-3,13-diazapentacyclo [11.8.0.0^{2,11}.0^{4,9}.0^{15,20}]henicosa-1(21),2,4(9),5,7,10,15(20)-heptaen-7-yl4-(piperidin-1-yl)piperidine-1-carboxylate[39,36].

4.1.5 Melting Point

222-223 ^{o}C

4.1.6 Molecular weight

586.6780

4.1.7 Solubility

Water soluble (1.07 g/ml)

4.1.8 Categories

Antineoplastic Agents

- Radiation-Sensitizing Agents
- Parasympathomimetics
- Enzyme Inhibitors
- Prodrugs
- Antineoplastic Agents, Phytogenic

4.1.9 Mechanism of action

Irinotecan inhibits the action of topoisomerase I. Irinotecan prevents religation of the DNA strand by binding to topoisomerase I-DNA complex. The formation of this ternary complex interferes with the moving replication fork, which induces replication arrest and lethal double-stranded breaks in DNA. As a result, DNA damage is not efficiently repaired and apoptosis (programmed cell death) occurs[39].

4.1.10 Pharmacology

Irinotecan is an antineoplastic enzyme inhibitor primarily used in the treatment of colorectal cancer. Irinotecan is a semisynthetic derivative of camptothecin. Camptothecins interact specifically with topoisomerase I, an enzyme in the cell nucleus that regulates DNA topology and facilitates nuclear processes

such as DNA replication, recombination, and repair. During these processes, topoisomerase I relieves torsional strain in DNA by inducing reversible single-strand breaks, allowing single DNA strands to pass through the break. The 3'-DNA terminus of the broken DNA strands bind covalently with the topoisomerase enzyme to form a catalytic intermediate called a cleavable complex.

After the DNA is sufficiently relaxed and the strand passage reaction is complete, DNA topoisomerase reattaches the broken DNA strands to form the chemically unaltered topoisomers that allow transcription to proceed. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent religation of these single-strand breaks. Current research suggests that the cytotoxicity of irinotecan is due to double-strand DNA damage produced during DNA synthesis when replication enzymes interact with the ternary complex formed by topoisomerase I, DNA, and either Irinotecan or SN-38. Mammalian cells cannot efficiently repair these double-strand breaks. The precise contribution of SN-38 to the activity of irinotecan in humans is not known. Irinotecan is cell cycle phase-specific (S-phase)[37].

4.1.11 Indication

For the treatment of metastatic colorectal cancer (first-line therapy when administered with 5-fluorouracil and leucovorin). Also used in combination with cisplatin for the treatment of extensive small cell lung cancer. Irinotecan is currently under investigation for the treatment of metastatic or recurrent cervical cancer.

4.1.12 Protein binding

30%-68%

4.1.13 Route of elimination

The cumulative biliary and urinary excretion of irinotecan and its metabolites (SN-38 and SN-38 glucuronide) over a period of 48 hours following administration of irinotecan in two patients ranged from approximately 25% (100 mg/m2) to 50% (300 mg/m2)[39].

4.1.14 Half-life

6-12 hours

4.1.15 Toxicity

Gastrointestinal complications, such as nausea, vomiting, abdominal cramping, diarrhea, and infection.

4.2 POLYMER PROFILE

PLGA is a synthetic copolymer of lactic acid and glycolic acid and is one of the most widely used FDA-approved biodegradable polymers for controlled release drug delivery systems. **PLGA** or **poly(lactic-*co*-glycolic acid)** is a copolymer which is used in a host of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility. PLGA is synthesized by means of random ring-opening co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. Common catalysts used in the preparation of this polymer include tin(II) 2-ethylhexanoate, tin(II) alkoxides, or aluminum isopropoxide. During polymerization, successive monomeric units (of glycolic or lactic acid) are linked together in PLGA by ester linkages, thus yielding a linear, aliphatic polyester as a product[7,86].

Depending on the ratio of lactide to glycolide used for the polymerization, different forms of PLGA can be obtained: these are usually identified in regard to the monomers' ratio used (e.g. PLGA 75:25 identifies a copolymer whose composition is 75% lactic acid and 25% glycolic acid). All PLGAs are amorphous rather than crystalline and show a glass transition temperature in the range of 40-60 °C. Unlike the homopolymers of lactic acid (polylactide) and glycolic acid (polyglycolide) which show poor solubilities, PLGA can be dissolved by a wide range of common solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate[7].

PLGA degrades by hydrolysis of its ester linkages in the presence of water. It has been shown that the time required for degradation of PLGA is related to the monomers' ratio used in production: the higher the content of glycolide units, the lower the time required for degradation. An exception to this rule is the copolymer with 50:50 monomers' ratio which exhibits the faster degradation (about two months). In addition, polymers that are end-capped with esters (as opposed to the free carboxylic acid) demonstrate longer degradation half-lives.

PLGA has been successful as a biodegradable polymer because it undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic acid. These two monomers under normal physiological conditions, are by-products of various metabolic pathways in the body. Since the body effectively deals with the two monomers, there is very minimal systemic toxicity associated with using PLGA for drug delivery or biomaterial applications. Also, the possibility to tailor the polymer degradation time by altering the ratio of the monomers used during synthesis has made PLGA a common choice in the production of a variety of biomedical devices such as: grafts, sutures, implants, prosthetic devices, micro and nanoparticles. It has also been used successfully in delivery Amoxicillin in treating listeriosis (treatment of Listeria monocytogenes infection)[2] As an example, a commercially available drug delivery device using PLGA is Lupron Depot for the treatment of advanced prostate cancer[66].

The biodegradation of PLGA occurs through a hydrolytic chain cleavage mechanism. In vivo, PLGA undergoes chemical hydrolysis as well as enzymatic cleavage of its backbone ester linkages to form the biologically compatible moieties, lactic acid and glycolic acid. The degradation products are subsequently eliminated from the body as carbon dioxide and water by the tricarboxylic acid cycle. The chemical structure of PLGA is illustrated in Figure , where “m” and “n” refer to the relative amounts of lactide and glycolide units, respectively, in a specific PLGA copolymer. The composition of PLGA can be varied by modifying the chain length (molecular weight), as well as the ratio of lactic to glycolic acid monomers in the polymer chain. This flexibility of composition is advantageous as it can be

manipulated to yield appropriate physical properties for a particular application. For example, the in vivo degradation rate of PLGA can be tailored by controlling the ratio of "m" and "n", with slower degradation rates observed for polymers with higher m/n ratios[56].

Although a number of different polymers have been investigated for formulating biodegradable nanoparticles, poly (l-lactic-acid) (PLA) and its copolymers with glycolic acid (PLGA) have been extensively used for controlled drug delivery systems[63,57]. The lactide/glycolide polymers chains are cleaved by hydrolysis into natural metabolites (lactic and glycolic acids), which are eliminated from the body by the citric acid cycle. PLGA provides a wide range of degradation rates, from months to years, depending on its composition and molecular weight[43,57].

PLGA has a long history of safe use as surgical sutures and implants, and it is applied in at least 12 different marketed products from 10 different companies worldwide. PLGA is used not only as a resorbable suture material and a scaffold for tissue engineering, but also in drug delivery. PLGA delivery platforms have been developed for the sustained and targeted delivery of plasmid DNA; recombinant HIV envelope (env) protein (***Moore et al., 1995)***; hormones ***(Sun et al., 2008)*** and anticancer agents. PLGA nanoparticles have been formulated as colloidal carrier systems to improve drug efficacy[21,22]**.** Drug release from PLGA nanoparticles is controllable through the rate of drug diffusion in the polymer matrix and/or degradation of the polymer matrix.

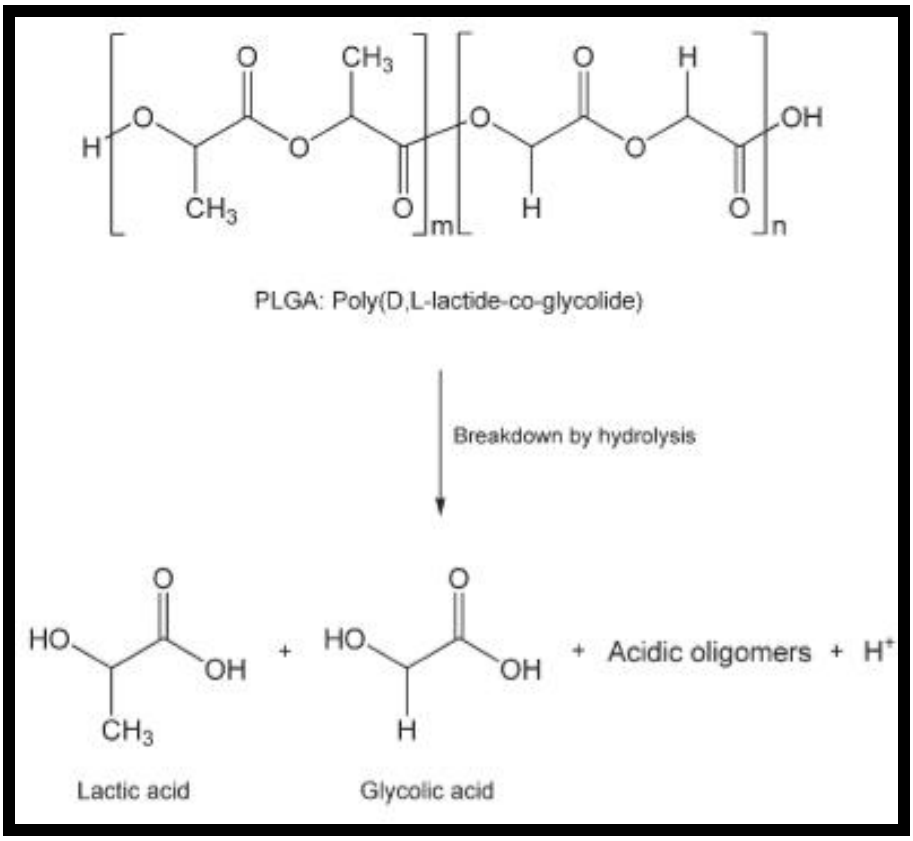

Figure 4.2: PLGA molecular structure

4.2.1 Method of Preparation

Preparation of nanoparticles is frequently based on the use of dispersed systems in which solid or liquid phases are dispersed in fluid media to constitute embryos of the final particles. Single and multiple emulsion systems have been used to encapsulate drugs into polymeric particles. Normally, an organic solvent is required to dissolve the polymers in the emulsion step. To decrease droplet size and avoid droplet coalescence, surfactants are usually required[56].

Techniques for preparing PLGA nanoparticles may be divided into bottom-up and top-down approaches. The bottom-up techniques, which include emulsion or microemulsion polymerization, interfacial polymerization and precipitation polymerization, employ the monomers as a starting point, and nanoparticle fabrication occurs simultaneously with monomer polymerization into PLGA. Top-down techniques include the emulsion evaporation, emulsion diffusion, solvent

displacement and salting out methods in which nanoparticles are prepared from preformed PLGA.

Of the techniques reported, emulsion evaporation and emulsion diffusion are the two most frequently employed. In the emulsion diffusion method, the PLGA polymer is dissolved in an organic phase which is partially miscible in water, and the organic phase is emulsified with an aqueous surfactant solution under stirring or sonication[67].

Diffusion of the organic solvent and the counter diffusion of water into the emulsion droplets induce polymer nanoparticle formation. The emulsion evaporation method is similarly based on emulsification principles, except that the organic solvent for dissolving the polymer/drug is highly volatile, and is evaporated off following emulsification. Examples of appropriate solvents include ethyl acetate, chloroform and methylene chloride. Like the emulsion diffusion method, the aqueous phase also contains a dissolved surfactant to impart stability, and emulsification is carried out under high-shear stress to reduce the size of the emulsion droplet. Important parameters in emulsion evaporation method that may affect nanoparticle size are: PLGA co-polymer ratio, polymer concentration, solvent characteristics, surfactant/polymer molecular mass, viscosity, phase volume ratios, stirring rate, and temperature[87,7].

4.2.2 Applications

Biodegradable nanoparticles formulated from PLGA have been widely investigated for sustained and targeted delivery of therapeutic agents, including plasmid DNA, proteins, and low molecular weight compounds. Encapsulation in PLGA nanoparticles could protect susceptible protein therapeutic agents from physical denaturation and chemical degradation, thereby lengthening their half-life. The administration route for the proteins can also be changed from injection, which is invasive and associated with poor patient compliance, to a more acceptable non-invasive route. PLGA nanoparticles are also extensively applied for the delivery of anticancer agents due to their capacity to enhance drug efficacy by regulating the

drug release rate, improving drug stability and prolonging drug circulation time *in vivo*. Incorporation of anticancer agents in nanoparticles has been shown to result in a more favorable circulation half-life, area under the concentration-time curve (AUC) and peak plasma concentration[86,14,45].

An example is the paclitaxel-loaded PLGA nanoparticles, which not only reduced the dose and frequency of administration of paclitaxel, but also provided improved targeting of the drug to cancer cells. Other anticancer agents investigated with PLGA nanoparticles included doxorubicin, camptothecin and 5-fluorouracil. Nevertheless, PLGA nanoparticles are not without shortcomings, one of which is their non-site specific targeting capability. Another is the rapid removal of the naked PLGA nanoparticles from the bloodstream by the RES system. Complete clearance takes only a matter of minutes, with most of the nanoparticle dose concentrating in the liver and spleen. Several approaches to improve the selectivity of the delivery system have been investigated, the most common of which is the application of nanoparticles conjugated with targeting ligands. Ligand conjugation allows the specific delivery of the drug load to cancer cells, which not only increases therapeutic efficacy, but also reduces side effects associated with non-discriminatory drug deposition.

In one study, monoclonal antibody (mAb)-modified PLGA nanoparticles were shown more likely to be bound to the targeted invasive epithelial breast tumor cells than non-coated nanoparticles[92]. PLGA nanoparticles functionalized with the A10 RNA aptamer also resulted in enhanced nanoparticle delivery to the prostate tumor in the xenograft mouse model compared to equivalent non-conjugated nanoparticles. In yet another study, PLGA nanoparticles conjugated with poly (L-lysine)-poly (ethylene glycol)-folate were observed to improve cellular uptake of the nanoparticles into the folate receptor over-expressing KB cells by folate receptor-mediated endocytosis. A few PLGA-based drug delivery formulations have been in use in the clinic. Lupron Depot, an injectable suspension formulation of leuprolide acetate-loaded PLGA microspheres, is the first peptide formulation for the treatment of advanced prostate cancer. Peptide release occurs over a period of 1, 3 or 4 months, and is governed mainly by the PLGA degradation rate. Nutropin DepotTM

is another PLGA microsphere formulation, and the FDA has approved it in late 1999 for the treatment of pediatric growth hormone deficiency. Consisting of micronized particles of recombinant human growth hormone (rhGH) embedded in a PLGA matrix, this formulation requires only one or two doses a month compared to the conventional therapy that requires multiple doses per week. There are as yet no PLGA nanoparticle-based drug delivery formulations in the market[89].

CHAPTER 5

ANALYTICAL METHOD DEVELOPMENT

5.1 MATERIALS

Materials	Suppliers
Irinotecan hydrochloride trihydrate	Zydus Research Centre
Acetonitrile	Merck specialities pvt ltd, Mumbai
Acetone	Merck specialities pvt ltd, Mumbai

Table 5.1 List of materials

5.2 CALIBRATION CURVE OF IRINOTECAN IN ACETONITRILE

A stock solution of Irinotecan in ACN was prepared by dissolving 5 mg of the drug in 50 ml of ACN. A serial dilution of stock solution was done to prepare various strength of IRN solution and the absorbance was measured using UV Visible spectrophotometer (UV-1700, Pharmaspec, Shimadzu, Japan) at wavelength of 256 nm. A calibration plot of absorbance vs. concentration was plotted.

5.3 CALIBRATION CURVE OF IRINOTECAN IN PHOSPHATE BUFFER SALINE (PBS pH 7.4)

A stock solution of Irinotecan in PBS was prepared by dissolving 5 mg of the drug in 50 ml of PBS. A serial dilution of stock solution was done to prepare various strength of IRN solution and the absorbance was measured using UV Visible spectrophotometer (UV-1700, Pharmaspec, Shimadzu, Japan) at wavelength of 256 nm. A calibration plot of absorbance vs. concentration was plotted.

5.4 RESULTS AND DISCUSSION

The calibration curve of irinotecan hydrochloride trihydrate was carried out in acetonitrile and phosphate buffer saline.

5.4.1 Calibration curve of IRN in ACN

Concentration	Mean Absorbance
0	0
10	0.708
20	1.455
30	2.105
40	2.731
50	3.221

Table 5.2: data of calibration curve of IRN in acetonitrile at 256 nm

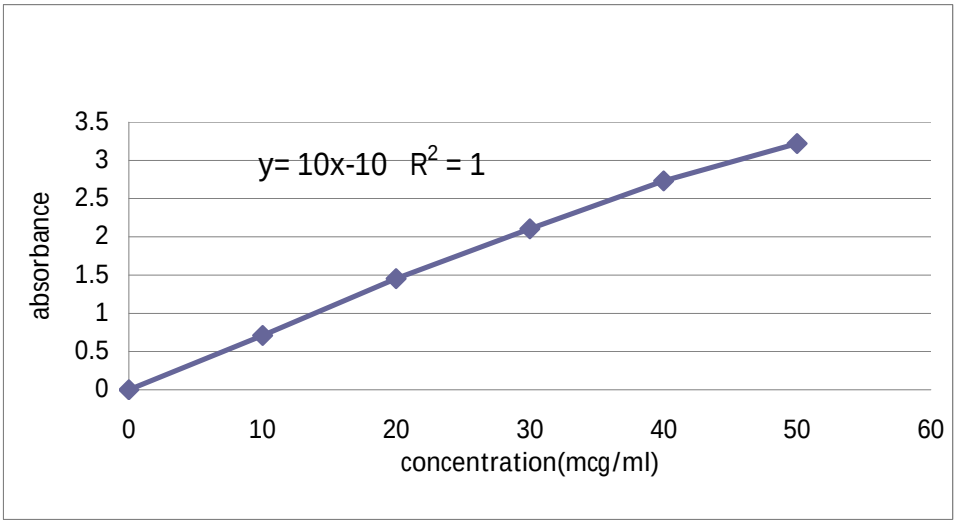

Figure 5.1: calibration curve of IRN in acetonitrile at 256 nm

The λmax in acetonitrile was 256 nm and the Beer- Lambert law was followed in the range 0.5 to 2.5 mcg/ml concentration.

5.4.2 Calibration curve of IRN in Phosphate buffer saline pH 7.4

Concentration	Mean Absorbance
0	0
5	0.558
10	0.866
15	1.308
20	1.726
25	2.232

Table 5.3 data of calibration curve of IRN in phosphate buffer pH 7.4 at 256 nm

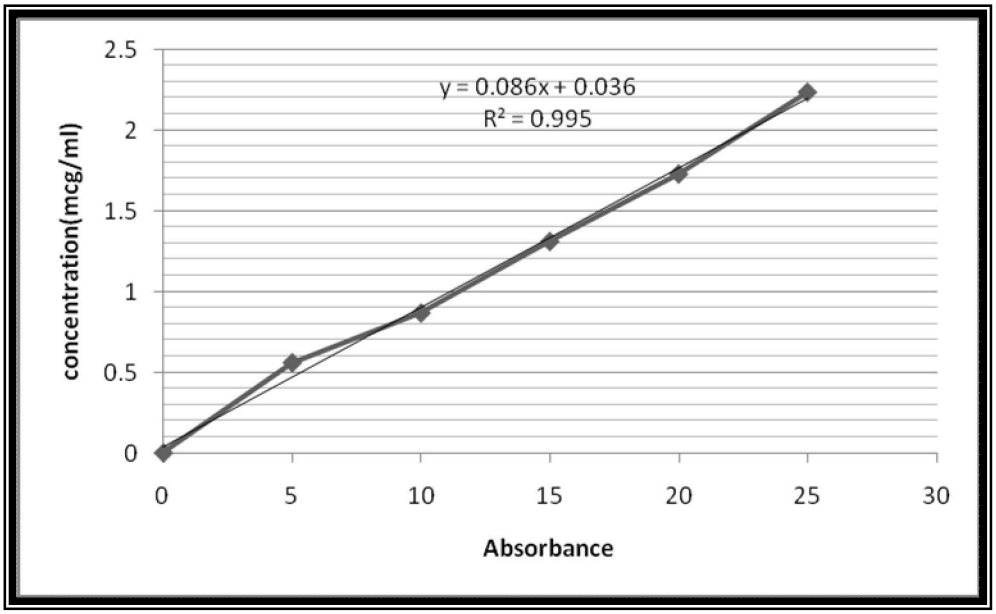

Figure 5.2-calibration curve of IRN in phosphate buffer pH 7.4 at 256 nm

The λmax in phosphate buffer saline pH 7.4 was at 256 nm and the Beer-Lambert law was followed in the range 0.5 to 2.5 mcg/ml concentration. Moreover we observed regression coeffecient value of 0.990.

5.5 POWDER X-RAY DIFFRACTION

Irinotecan hydrochloride was characterized by its powder X-ray diffraction pattern,comprising 2θ angle values of about 7.60; 8.30; 9.55; 11.00; and 12.40. The Relative Intensity (%) of the mentioned characteristics reflection peaks of Form b at the 2θ angle values are reported in table.

Angle 2θ	Relative intensity %
7.60	47.9
8.30	33.4
9.55	36.9
11.00	97.3
12.40	88.1

Table 5.4 X-Ray diffraction pattern

Polymorphism' is the property of some molecules to adopt more than one crystalline form in the solid state. A single molecule can give rise to a variety of solids having distinct physical properties that can be measured in a laboratory like its thermal behavior, e.g. melting point and differential scanning calorimetry ("DSC") thermogram, dissolution rate, flowability, X-ray diffraction pattern, infrared absorption spectrum and NMR spectrum. The differences in the physical properties of polymorphs result from the orientation and intermolecular interactions of adjacent molecules in the bulk solid. Accordingly, polymorphs are distinct solids sharing the same molecular formula which can yet have distinct advantageous and/or disadvantageous physical properties compared to other forms in the polymorph family.

One property of a pharmaceutical compound that can vary depending upon its polymorphic form is its rate of dissolution in aqueous solvent. The rate of dissolution can have therapeutic consequences since it can affect the rate that an orally administered pharmaceutical is delivered to the bloodstream of a patient.

CHAPTER 6

FORMULATION DESIGN OF IRINOTECAN HYDROCHLORIDE TRIHYDRATE NANOPARTICLES

6.1 LIST OF EQUIPMENT

Equipments	Manufacturers
Micropipettes (tripett)	10µl ,1000µl, 100µl Ependorff ,Germany
Sonicator	PRAMA Ultrasonic cleanser, Mumbai
U. V. Spectrophotometer	Shimadzu UV-1601, Japan
Zeta sizer	Malvern (Amili Ltd.), United Kingdom
Electronic Balance	Mettler Toledo (Ag135), United states
Mettler Toledo electronic balance (For weight less than 100mg)	Mettler Toledo (Ag135), United states
Centrifuge	REMI Equipment, Delhi
Hot air oven	Scientific equipment, India
Digital weighing balance	Mettler Toledo, United States
Magnetic stirrer	Tarsons spinot digital, Germany

6.2 MATERIALS

Materials	Suppliers
Irinotecan hydrochloride trihydrate	Zydus Research Centre, Ahemdabad
Poly(dl-lactide-co-glycolide)(50:50)	Durect corporation, Birmingham division, Pelham
Acetonitrile	Merck specialities pvt ltd, Mumbai
Acetone	Merck specialities pvt ltd, Mumbai
Poloxomer 188	Sigma chemical co., St Louis,USA
Poly vinyl alcohol	Sigma chemical co., St Louis,USA
Sodium sulphate	Canton laboratories pvt, ltd, Baroda, India
Sodium chloride	Canton laboratories pvt, ltd, Baroda, India

Table 5.1 : list of materials

6.3 FORMULATION OF IRN NANOPARTICLES

6.3.1 Nanoparticle preparation

a) Nanoparticles were prepared by using solvent evaporation method. Polymer and drug was firstly dissolved in organic phase.

b) Then a weighed quantity of surfactant was dissolved in aqueous phase.

c) Then the organic phase was added to aqueous phase in a dropwise manner.

d) The suspension was kept on mechanical stirring until the complete evaporation of solvent.

e) The residual quantity of solvent was removed by rotatory vacuum evaporator for 1 hour.

f) Then this dispersion was passed through the Sephadex G-25 column (size exclusion chromatography) for the separation of free drug and entrapped drug[89].

Preparation of organic phase: The required amount of the organic solvent was measured and was taken in a 10 ml beaker. Then drug and polymer was separately weighed and was dissolved in organic phase.

Preparation of aqueous phase: the required amount of selected surfactant was dissolved in water.

Serparation through Sephadex G-25 column*:* The sephadex G- 25 column was used to separate free drug and entrapped drug. This separation follows size exclusion chromatography. In this separation an aliquot of nanoparticulate dispersion is passed through the sephadex G-25 column. Firstly the free drug i.e. drug soluble in aqueous phase is separated as a transparent solution through the column. Then the nanoparticulate suspension comes out of the column as a bluish white suspension which is collected separately. The reason for this is that the internal pores of the sephadex G-25 column will adjust the the paticles in size range of 10 – 500nm. Hence the nanoparticles will be entrapped in the pores of column while the bigger

particle size free drug will flow out through the at first succeeded by nanoparticles which can be collected and further characterization can be performed.

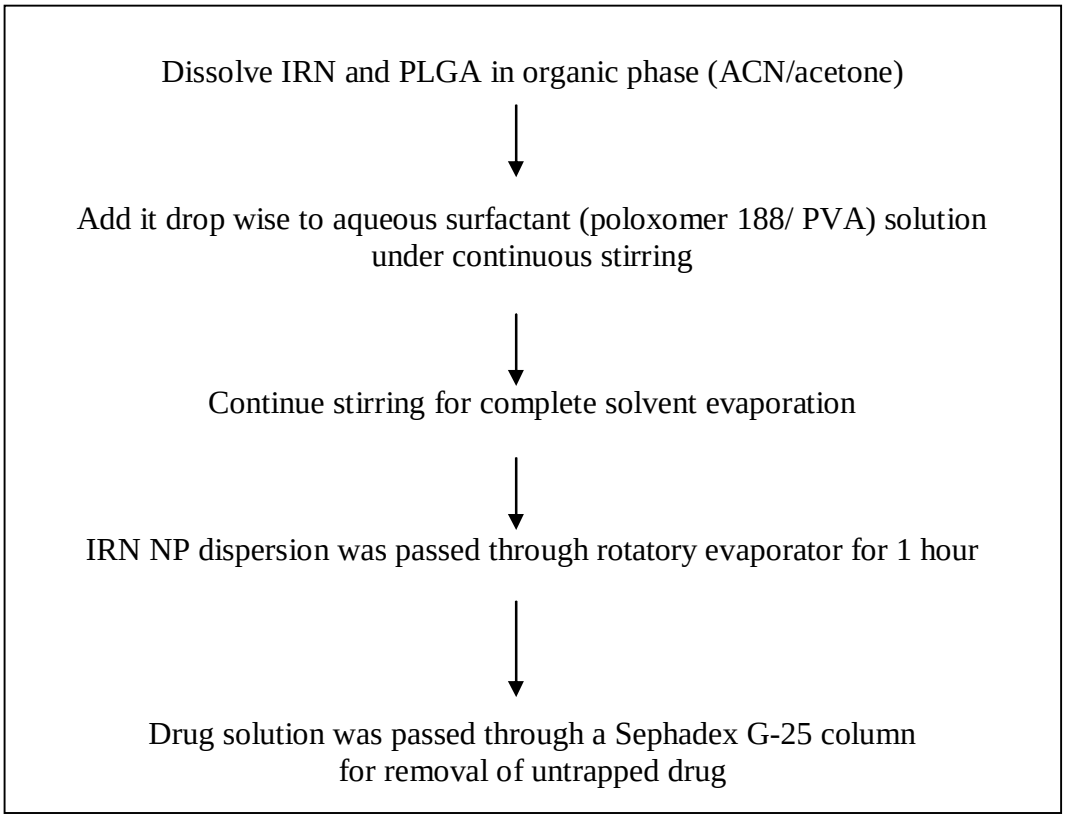

Figure 6.1 Process Flow chart

6.3.2 Formulation optimization

The aim of this work is to optimize and characterize the formulation prepared by solvent evaporation method for the preparation of nanoparticles in order to identify formulation and process parameters.

6.3.2.1 Process parameters

6.3.2.1.1 Selection of speed of the magnetic stirrer

a) The speed of magnetic stirrer at which nanoparticles are prepared affects the particle size and percent drug entrapment due to different evaporation rate.

b) Different batches were prepared at various stirring speed of 400, 600 and 800 rpm and its effect on the nanoparticles preparation was studied.

c) The particle size, PDI and percent drug entrapment were evaluated.

6.3.2.1.2 Effect of rate of addition of organic phase to aqueous phase:

a. The rate of addition of the organic phase to the aqueous phase governs the formation of nanoparticles.

b. The speed at which the solvent is added will demonstrate its effect on the formulation[3].

c. For this purpose the rate of addition of solvent containing drug and polymer to aqueous surfactant solution was kept at 0.25ml/min, 0.5ml/min and 1ml/min.

d. The effect of solvent addition rate on the particle size, PDI and percent drug entrapment was evaluated.

6.3.2.2 Formulation parameters

6.3.2.2.1 Solvent selection (organic phase)

a. The selection of organic solvent for preparation of nanoparticles by solvent evaporation method is very essential.

b. So before formulation of nanoparticles one must have to select the proper solvent in which the drug is maximum soluble in the range which is essential in the nanoparticle drug delivery system[3].

c. Acetonitrile and acetone are the most common of the solvents reported for preparation of nanoparticles.

d. Both the solvents were used initially at ratio of 1:2 ratio of nanoparticulate system in aqueous phase keeping other parameters constant.

e. The effect of these solvents on particle size, PDI and percent drug entrapment was evaluated.

f. The IRN is insoluble in acetone, so it was dissolved in aqueous phase and the nanoparticles were fabricated in ACN.

6.3.2.2.2 Surfactant Selection

a. Surfactant is a substance that reduces surface tension between the two phases. Surfactant also aid in improving drug stability.

b. For formulating nanoparticle drug delivery system the stability of the drug in different surfactants is an essential step.

c. So before formulation of nanoparticles one must have to select the proper surfactant, which is essential in the nanoparticle drug delivery system[4].

d. In order to optimize the concentration of aqueous surfactant solution in IRN NP, the nanoparticles were prepared by using PVA and poloxomer188 concentrations of 1% to 2 % each and other parameters were kept constant and their effect on particle size, PDI and percent drug entrapment was evaluated.

6.3.2.2.3 Effect of drug to polymer ratio on formulation

a) Drug to polymer ratio also plays a vital role in optimization of a formulation.

b) The IRN NP with the different ratios of drug/PLGA i.e., 1:5, 1:10 and 1:20, were prepared keeping other parameters constant.

c) The amount of drug was kept constant while amount of polymer was varied. The particle size, PDI and percent drug entrapment were evaluated.

6.3.2.2.4 Effect of aqueous to organic phase ratio on formulation:

a) The aqueous to organic phase ratio in the nanoparticle formation was also optimized, varying the amount of organic phase in three different formulation batches and keeping the amount of aqueous phase constant.

b) Here PLGA and drug were weighed and both drug and polymer were dissolved in the 10ml (1:1), 5ml (1:2), 3.33 (1:3), 2.5ml (1:4) acetonitrile for 4 different batches respectively.

c) The particle size, PDI and percent drug entrapment were evaluated.

6.3.2.2.5 Effect of poloxomer188 concentration on formulation:

a) The presence of surfactant molecules stabilizes the emulsion nanodroplets and prevents them from coalescing with each other[3].

b) In order to optimize the concentration of aqueous surfactant solution, the IRN NP were prepared by using poloxomer-188 at various concentrations of 1%, 2%, 3% and 4% each and other parameters were kept constant.

c) The effect of poloxomer188 on particle size, PDI and percent drug entrapment was studied.

6.3.2.2.6 Effect of salt addition on formulation:

a) Two salts i.e. sodium sulphate and sodium chloride were used in various concentrations i.e. 2%, 1%, 0.5% and 0.1%.

b) These salts were added in the aqueous phase along with the surfactant. The effect of salt addition of IRN NP on the particle size, PDI and percent drug entrapment was evaluated.

6.3.3 Characterization of IRN nanoparticles

6.3.3.1 Percent drug entrapment

a) An aliquot of IRN NP dispersion was added to CAN and sonicated well to dissolve nanoparticles completely.

b) The absorbance of the solution was measured at λmax of 256nm using U.V. visible spectrophotometer (UV-1700, Pharmaspec, Shimadzu, Japan).

c) The percent drug entrapment was calculated using following formula.

$$\textbf{Drug entrapment (\%, w/w)} = \frac{\textbf{Amount of drug in nanoparticles}}{\textbf{Total amount of the drug used}} \times \textbf{100}$$

6.3.3.1 Measurement of particle size and zeta potential:

a) The mean particle size, polydispersity index and zeta potential of prepared IRN NP was measured using dynamic light scattering method.

b) Briefly the IRN NP dispersion was filled in the cuvette and placed in the zeta sizer (Nano 25, Malvern, UK).

c) Analysis was performed at 25 °C with an angle of detection of 90°.

d) Each reported value is the average of three measurements.

e) Each measurement was performed in triplicate and particle size, PDI and zeta potential was measured.

6.3.3.4 Lyophilization of the IRN NP:

a) Here two cryoprotectants i.e. Sucrose and Trehalose were used at different ratio of solid content to cryoprotectant.

b) The ratio (w/w) of total solid content to cryoprtectant was selected from 1:3, 1:5 and 1:7.

c) The cryoprotectants were dissolved in the IRN NP dispersion as per different ratio and the vials were lyophilized using lyophilizer Vertis Advantage, USA.

d) The total time for lyofilization was kept 36hrs.

e) After lyophilization, vials were removed and sealed immediately.

f) The lyophilized vials were reconstituted with 3ml of d.m. water followed by 2 min bath sonication and particle size and PDI was measured using zeta sizer (Nano ZS, UK).

6.3.3.5 Description of in vitro release process for IRN NP:

a. The in vitro drug release study for IRN NP was carried out using dialysis method. Briefly the IRN NP dispersion equivalent to 1.5mg of IRN was placed in dialysis tube of mol. wt 12,000 (Sigma Aldrich, Mumbai).

b. The tube was sealed and dipped in a beaker 500ml of phosphate buffer saline pH 7.4.

c. The buffer medium was stirred at speed of 100 rpm and the temperature of medium was kept 37 ± 2^0 C.

d. After certain time intervals, 3ml samples were withdrawn and the media was replenished with same volume of fresh buffer using UV visible spectrophotomer (UV-1700, Pharmaspec, Shimadzu, Japan) and the percent cumulative drug release was calculated based on calibration curve of IRN in PBS pH 7.4 (chapter3 section 3.3)

CHAPTER 7

RESULT AND DISCUSSION

7.1 SELECTION OF SPEED OF THE MAGNETIC STIRRER:

The stirring speed of the mechanical stirrer for preparation of IRN NP was kept at various rpm i.e. 400, 600, 800. The effect of stirring speed on the particle size, PDI and percent drug entrapment was evaluated and are shown in table 4.1.

Batch code	Rotation per minute	Particle size (nm)	Poly dispersivity index	Percent drug entrapment
IRNF024	800	211.6±10.4	0.154±0.020	30
IRNF025	600	218.3±12.5	0.116±0.023	33.82
IRNF026	400	336.5±14.7	0.302±0.019	26.082

Table 7.1 Effect of rotation speed

a) The water miscibility of the solvent is determining factor for nanosuspension preparation.

b) At 600 rpm, high solubility of acetonitrile in water enables their fast diffusion from dispersed droplets into aqueous phase.

c) Thus, as soon as the dispersed phase comes in contact with a large amount of aqueous phase during the emulsion dilution, fast diffusion of organic solvent occurs, leading to fast drug precipitation and particle formation.

d) The finding obtained confirms that at higher speed (i.e 800 rpm) less particle aggregation occurs but the PDI observed was very high

compared to batch prepared at 600 rpm, which having similar particle size with lower PDI.

e) Furthur at speed of 400rpm a aggregation was observed and resulted into higher particle size[3].

Hence the stirring speed was optimized to 600 rpm.

7.2 EFFECT OF RATE OF ADDITION OF ORGANIC PHASE TO AQUEOUS PHASE

a) The rate of addition of solvent containing drug and polymer to aqueous surfactant solution was varied to 0.25ml/min, 0.5ml/min and 1ml/min.

b) The effect of solvent addition speed on the nanoparticles preparation was studied and following results were obtained.

Batch code	Rate of addition	Particle size (nm)	Poly dispersivity index	Percent drug entrapment
IRNF027	0.25 ml/min	309.6±10.8	0.272±0.020	26
IRNF028	0.50ml/min	213.0±9.6	0.113±0.023	35.3
IRNF029	1 ml/min	245.6±12.1	0.192±0.024	21.3

Table 7.2 effect of rate of addition

c) The rate of addition of the organic phase to the aqueous phase governs the formation of nanoparticles.

d) The speed at which the solvent is added will demonstrate its effect on the formulation.

e) As shown in table 4.2, the rate of addition of organic phase at rate of 0.5 ml/min resulted into optimum particle size with lower PDI.

f) In addition, the percent drug entrapment was higher compared to other batches.

Hence from the above results it was clear that rate 0.5ml/min was the optimum speed for the addition of the organic phase to aqueous phase.

7.3 SOLVENT SELECTION

a) For formulating nanoparticle drug delivery system the solubility of the drug in different solvents is an essential step.

b) So before formulation of nanoparticles one must have to select the proper solvent in which the drug is maximum soluble in the range which is essential in the nanoparticle drug delivery system[3].

c) In literature, various solvent lire CAN, DMSO, DMF, ethyl acetate were used for preparation of PLGA NP's.

d) Out of these two solvents are acetone and acetonitrile, which are commonly reported in the literature for formation of PLGA NP's.

Batch code	Solvent	Particle size (nm)	Poly dispersivity index	Percent drug entrapment
IRN F002	Acetone	336.1±12.3	0217±0.010	11.4
IRN F003	Actonitrile	223.2±10.3	0.113±0.014	34.2

Table 7.3 Effect of solvent

e) As shown in table 4.3 it was observed that IRN nanoparticles prepared using acetonitrile showed lower particle size and PDI and had comparatively higher percent drug entrapment than acetone.

f) In acetone lower drug entrapment was observed because drug was dissolved in aqueous phase for the preparation of IRN NP.

Hence acetonitrile was selected was a solvent of choice for nanoparticle preparation.

7.4 SURFACTANT SELECTION

a) In order to optimize the concentration of aqueous surfactant solution, the nanoparticles were prepared by using PVA and Poloxomer188 concentrations of 1% to 2 % each and other parameters were kept constant and results obtained are shown in table 4.4

b) Surfactant is a substance, which reduces surface tension between the two phases. Surfactant also aid in improving drug stability.

c) For formulating nanoparticle drug delivery system the stability of the drug in different surfactants is an essential step.

d) So before formulation of nanoparticles one must have to select the proper surfactant, which is essential in the nanoparticle drug delivery system.

e) The presence of surfactant molecules stabilizes the emulsion nanodroplets and prevents them from coalescing with each other.

Batch code	Surfactant	Particle size (nm)	Poly dispersivity index	Percent drug entrapment	Remarks
IRNF004	1% PVA	-	-	-	Aggregation observed
IRNF005	2%PVA	-	-	-	Aggregation observed
IRNF006	1% Poloxomer188	445.3±11.4	0.342±0.010	25.3	Passed
IRNF007	1% Poloxomer188	218±10.2	0.108±0.013	33.82	Passed

Table 7.4 Surfactant selections

f) For effective stabilization, the surfactant molecules must cover the organic/aqueous interfacial area of all the droplets[3].

g) Hence a minimum number of surfactant molecules are required to achieve small particle size and narrow size distribution.

h) As shown in table 4.4, the batches prepared using PVA showed aggregation upon solvent evaporation.

Hence 2% of poloxomer-188 was selected as the surfactant of choice for nanoparticle preparation.

7.5 DRUG: POLYMER (D: P) RATIO

a) Drug to polymer ratio also plays a vital role in optimization of a formulation[4].

b) The nanoparticles of IRN with the different ratios of drug: PLGA i.e., 1:5, 1:10 and 1:20, were prepared using solvent evaporation method.

c) The amount of drug was kept constant while amount of polymer was varied and results obtained are shown in table 4.5.

Batch code	D:P Ratio	Particle size (nm)	Poly dispersivity index	Percent drug entrapment	Remarks
IRNF008	1:5	190.3±8.3	0.271±0.013	8.476	Failed
IRNF009	1:10	223.1±8.2	0.079±0.018	37.2	Passed
IRNF010	1:20	323.2±12.6	0111±0.010	6.32	Failed

Table 7.5 Effect of drug to polymer ratio

d) From the above observations, it observed that on decreasing the drug to polymer ratio the particle size increased with decrease in the percent drug entrapment.

e) At 1:10 ratio of polymer an optimum particle size and percent drug entrapment was observed.

Hence as a result the drug to polymer ratio was selected as 1:10.

7.6 AQUEOUS: ORGANIC PHASE RATIO

a) The aqueous to organic phase ratio was varied to note its effect on the particle size and percent drug entrapment Results obtained are shown in table 4.6.

Batch code	Aq:Or Ratio	Particle size (nm)	Poly dispersivity index	Percent drug entrapment	Remarks
IRNF011	1:1	-	-	-	Aggregation was observed
IRNF012	1:2	232.1±4.2	0.111±0.030	32.4	Passed
IRNF013	1:3	268.7±10.2	0.116±0.025	31.7	Passed
IRNF014	1:4	281.8±11.7	0.173±0.021	30.7	Passed

Table 7.6 Effect of aqueous to organic phase ratio

b) From the above results obtained, it was noted that with decrease in the volume of organic phase, resulted in the increase in particle size and PDI of IRN NP.

c) A decrease in the percent drug entrapment was also noted.

d) However, we observed no difference in percent drug entrapment upon changing ratio of aqueous to organic phase.

e) At 1:2 ratio we observed less particle size with low PDI.

Hence the aqueous to organic phase ratio was selected as 1:2.

7.7 POLOXOMER188 CONCENTRATION

a) In order to optimize the concentration of aqueous surfactant solution, the nanoparticles were prepared by using poloxomer-188 at concentrations of 1%, 2%, 3% and 4% and other parameters were kept constant. Results were obtained is summarized in the table 4.7.

Batch code	Poloxomer188 concentration	Particle size (nm)	Poly dispersivity index	Percent drug entrapment
IRNF015	1%	292.4±9.2	0.372±0.014	16.62
IRNF016	2%	221.3±10.6	0.172±0.010	35.64
IRNF017	3%	239.4±13.7	0.297±0.016	27.56
IRNF018	4%	243.6±10.3	0.201±0.013	25.23

Table 7.7 Effect of poloxomer 188 concentration

b) As the Poloxomer188 concentration is increased, the mean diameter of nanoparticles increased.

c) As shown in table 4.7, it was observed that there was no any major change in the particle size, but the PDI and percent drug entrapment was altered.

d) The use of poloxomer188 at 3% & 4% concentration showed no benefit compared to 2% poloxomer 188[3].

Hence the poloxomer 188 concentration was selected as 2%.

7.8 SALT ADDITION

a) In order to increase percent drug entrapment two salts were used i.e. sodium sulphate and sodium chloride in varying concentrations i.e. 2%, 1%, 0.5% and 0.1%. Results obtained are shown in table 4.8.

Batch code	Salt concentration	Particle size (nm)	Poly dispersivity index	Percent drug entrapment	Remarks
IRNF018	Sodium sulphate 2%	-	-	-	No formation of nanoparticles took place
IRNF019	Sodium sulphate 1%	-	-	-	No formation of nanoparticles took place
IRNF020	Sodium sulphate 0.1%	247.9±20.3	0.397±0.012	23.87	Passed
IRNF021	Sodium chloride 1%	-	-	-	No formation of nanoparticles took place
IRNF022	Sodium chloride 2%	-	-	-	No formation of nanoparticles took place
IRNF023	Sodium chloride 0.1%	232.5±17.8	0.301±0.013	14.47	Passed

Table 7.8 Effect of salt addition

b) The use of both the salt resulted into increase in particle size with decrease in drug entrapment at 0.1% concentration, while the 1% and 2% concentration of these salts did not induced nanoprecipitation and IRN NP was notb formed.

c) Hence the use of salt i.e. NaCl and sodium sulphate showed no any role increase in drug entrapment with maintenance of particle size near to 200nm^3.

So no salts were added to the formulation.

7.9 IN VITRO RELEASE PROFILE OF IRN NP

The in vitro release pattern of IRN NP is represented in table 4.9 and figure 4.1

Time	Cumulative Percent drug release
15 min	0
30 min	0.4
1 hours	1.3
2 hours	4.75
4 hours	20.59
6 hours	31.85
8 hours	48.32
24 hours	87.3

Table 7.9 In vitro profile of IRN NP

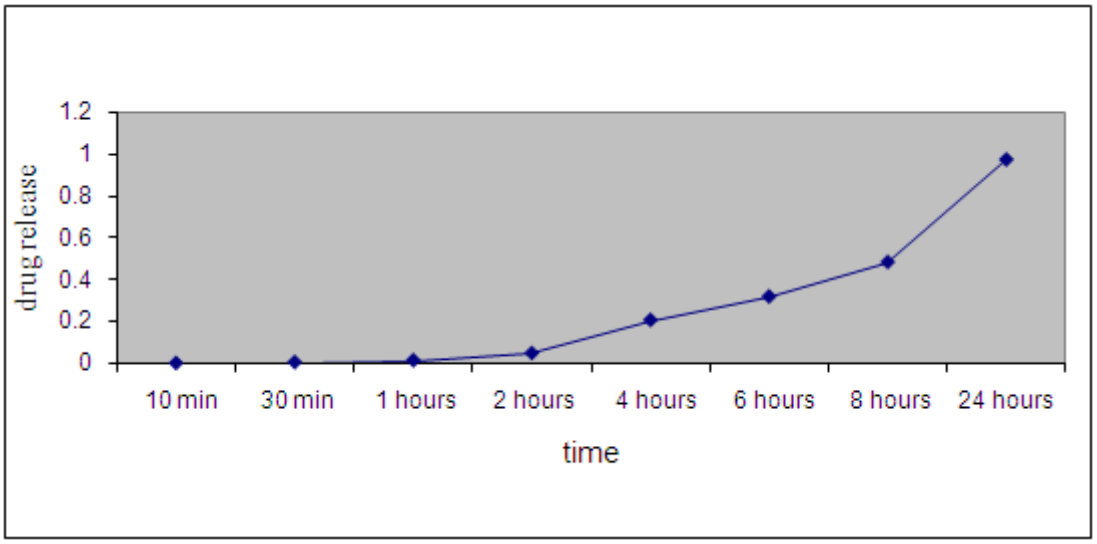

Figure 7.1 a plot showing in vitro release pattern of IRN NP

a) The drug release profile of IRN NP showed sustained release of drug over 24 hours.

b) A initial burst release of 20.59 was observed at 4hour followed by sustained release.

c) The results obtained are in similar fashion of other water-soluble drug entrapped in PLGA NP's[3].

7.10 LYOFILIZATION OF IRN NP

a) Lyophilized IRN NP with different cryoprotectant at various ratios were reconstituted with d.m. water and following data were obtained.

Batch code	Cryoprotectants	Particle size	PDI
IRNF030	Sucrose 1:3	367.1± 20.1	0.203±0.023
IRNF031	Sucrose 1:5	277.2± 10.2	0.227±0.025
IRNF032	Sucrose 1:7	284.2± 13.3	0.256±0.015
IRNF033	Trehalose1:3	215.5± 11.4	0.112±0.010
IRNF034	Trehalose1:5	186.1± 16.4	0.094±0.013
IRNF035	Trehalose1:7	198.1± 12.8	0.101±0.006

Table 7.10: Lyofilization of IRN NP

b) Trehalose as a cryoprotectant at ratio of 1:3 showed particle size after reconstitution near to the initial particle size.

c) The trehalose at higher ratio (1:5 and 1:7) showed minor change in particle size and PDI.

d) It was found that trehalose at 1:3 ratio showed comparatively better cryoprotective behavior to other ratio of trehalose and sucrose.

e) Trehalose seems to be a preferable cryoprotectant for biomolecules.

f) It has many advantages in comparison with the other sugars as: less hygroscopicity, an absence of internal hydrogen bounds which allows more flexible formation of hydrogen bonds with nanoparticles during freeze-drying, very low chemical reactivity and finally, higher glass transition temperature Tg′[52].

7.11 PARTICLE SIZE AND ZETA POTENTIAL MEASUREMENT:

a) The particle size and zeta potential was measured using zeta sizer (Nano ZS, Malvern, UK).

b) Zeta potential is the overall charge acquired by particles in a particular medium and its value gives the indication of potential physical stability of nanoparticles dispersion.

c) If all the particles have large positive or negative of zeta potential they will repel each other and system is considered to be stable.

d) Higher the value, more stable the system.

e) The zeta potential obtained was –13.3 mV. The particle size and zeta potential are shown in figure 4.2 and 4.3 respectively.

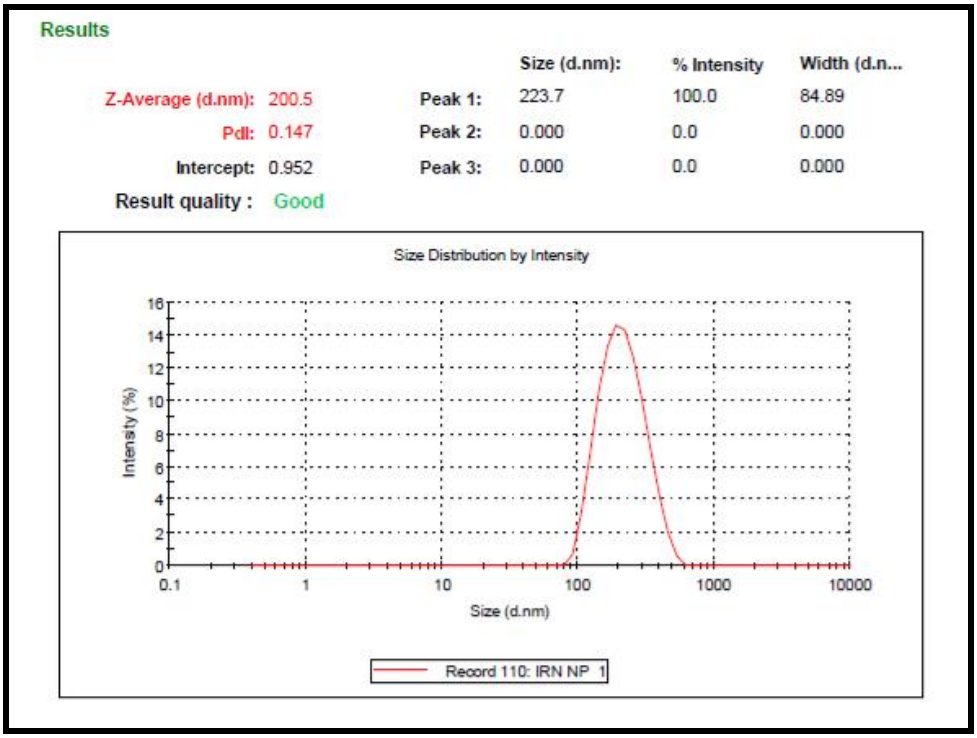

Figure 7.2 Particle size of IRN NP

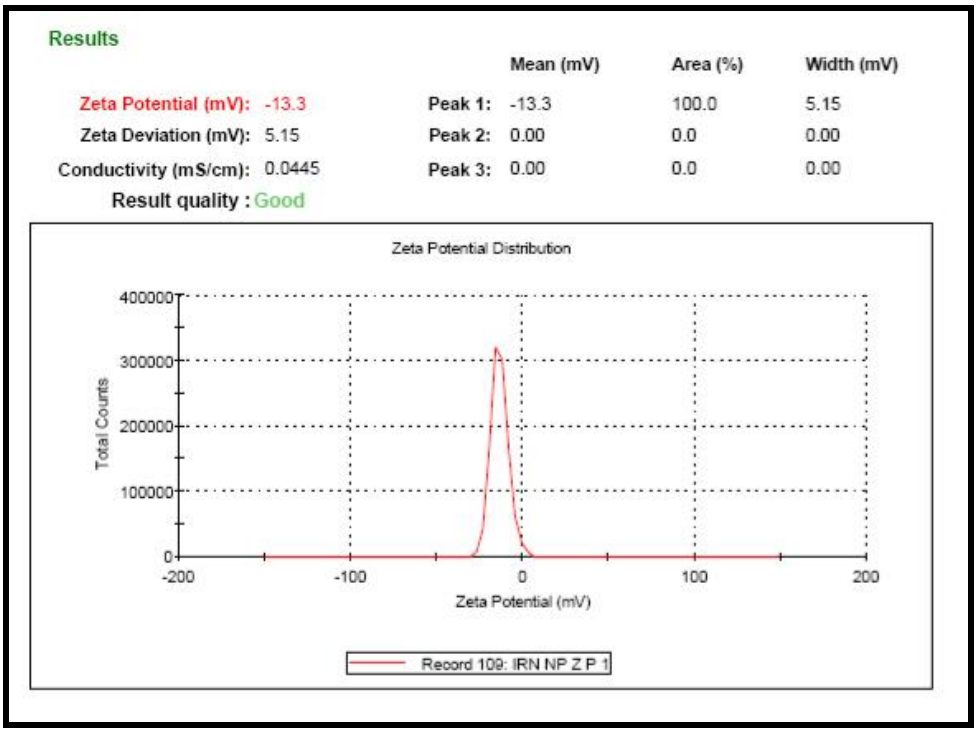

Figure 7.3 Zeta potential of IRN NP

CHAPTER 8

SUMMARY AND CONCLUSION

Summary

As the field of targeted drug delivery continues to move forward, it will be increasingly important to design nanoscale systems with tailorable properties for efficient delivery and improved therapeutic efficacy. Important design considerations will include the physicochemical properties that govern targeting, biodistribution, and clearance as well as the system's effectiveness in carrying, protecting, and even releasing active therapeutic and diagnostic agents. The formulation, optimization and characterization of irinotecan hydrochloride trihydrate nanoparticles was successfully carried out.

Conclusion

1. **The analytical method** developed for IRN in ACN and PBS pH 7.4 showed regression coeffecient near to 1 and followed Beer Lambert's law.
2. Irinotecan PLGA nanoparticles were prepared using **solvent evaporation method**.
3. **Formulation and optimization** of IRN NP was carried out using various formulation and process parameters.
4. **Solvent selection**: Acetonitrile was selected as the solvent of choice
5. **Surfactant selection**: Poloxomer 188 was selected as surfactant.

6. **Drug to polymer ratio**: The drug to polymer ratio was optimized to be 1:10

7. **Organic phase to aqueous phase ratio**: The organic phase to aqueous phase ratio was optimized as 1:2.

8. **Poloxomer 188 concentration**: The poloxomer188 concentration was selected as 2%.

9. **RPM of the magnetic stirrer**: The speed of rotation of the magnetic stirrer was kept 600 rpm

10. **Rate of addition of organic phase to aqueous phase**: The rate of addition of organic solvent to the aqueous solvent was 0.5 ml/min.

11. **Zeta potential**: Zeta potential is the overall charge acquired by particles in a particular medium and its value gives the indication of potential physical stability of nanoparticles dispersion. The zeta potential obtained was –13.3 mV.

12. **Percent drug entrapment**: The maximum percent drug entrapment was found out to be 37.2% of the batch IRN F009.

13. **In vitro drug release**: The in vitro drug release of IRN NP was also found out using dialysis method in phosphate buffer saline pH 7.4. The in vitro drug release showed sustained release of drug over 24 hours.

14. **Lyofilization**: Lyophilized IRN NP with different cryoprotectant at Svarious ratios of solid drug to cryoprotectants was used. Compared to sucrose, trehalose served as better cryoprotectant and maintained the original particle size.

Hence the IRN loaded PLGA nanoparticles have potential as a drug delivery system. Furthermore, they may have utility for site-specific drug delivery since the small size of the particles may allow their delivery to extra vascular target sites through the leaky endothelia of inflamed and cancerous areas.

CHAPTER 9

BIBLIOGRAPHY

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